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Farming striped catfish in Vietnam
Credit: Hoang Phan, Virbac, Vietnam

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Zuridah Merican

2020 - No Turning Back

It has been a disturbing 2020; initially firefighting supply chain disruptions and by May, learning about new routes to market and how to reach customers virtually. We found new ways of communication; virtual meetings to impart and gain knowledge, launch products and engage in interactive exchanges online to network across the globe. Imagine, FAO launched its SOFIA with more than 1,000 participants!

During the first wave of the pandemic, a serious breakdown in logistics occurred, arguably because authorities in many countries did not understand the aquaculture supply chain. During SAP's 2-day conference on global shrimp supply and demand, Paresh Kumar Shetty, Avanti Feeds, detailed the trauma faced by shrimp farmers and processing plants while Ravi Kumar Yellanki, Vaisakhi BioMarine, emphasised how global the farmed shrimp supply chain is, i.e. broodstock from the Americas and final products to markets in US, China and Europe. Hatcheries only bounced back into production with the arrival of broodstock via cargo and charter flights arranged by hatchery operators (see pages 4-5). In April, virtual audits by certification bodies were being explored so as not to disrupt exports.

OUR MISSION

We strive to be the beacon for the regional aquaculture industry.

We will be the window to the world for Asia-Pacific aquaculture producers and a door to the market for international suppliers.

We strive to be the forum for the development of self-regulation in the Industry.

Our editorial in July reviewed how this pandemic had forced the whole industry to look at its vulnerabilities. Brokers and wholesale markets were disrupted when movements were restricted. Online sales flourished via Facebook, Instagram and when online malls such as Lazada came forward to sell fresh seafood. The pandemic highlighted flaws in the market structure for high value marine fish where there were no plans for processing. Long before this pandemic, authorities in some countries stopped well-boats plying the region to collect fish for markets in China and Hong Kong. Now, good survival rates and excess supply have dropped grouper prices by as much as 40% in Malaysia. Demand from seafood restaurants which experienced intermittent closures or limited seating has been poor.

This pandemic has also impacted the demand for farmed shrimp. Leading analysts reported that the loss in demand from the foodservice sector was far deeper than the rise in retail sales in the US. Toward the end of the 2019, shrimp supply was already exceeding demand, sending prices on a downtrend. The forecast for farmed shrimp in 2020 varies from a reduction by 1.4 million tonnes according to Soraphat Panakorn (issue September/October) to 10.5% less than the 4 million tonnes in 2019 estimated at GOAL 2020.

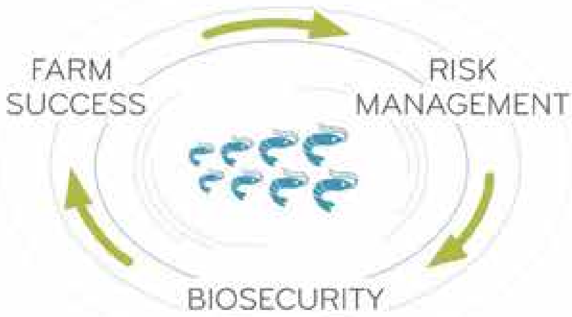
While the pandemic depressed vannamei shrimp prices, it remains relatively stable in countries where local consumption is important. In Malaysia, some 20% drop in prices for the monodon shrimp, mainly targeted for live markets in China and Singapore, reflected the difficulties in export logistics. Some 15% and 30% of farmed shrimp in Indonesia and Thailand, respectively, stay in local markets. India which exports 95% of its shrimp, is pivoting to the development of the local market.

Covid-19 brought about loss of incomes, particularly in Asia where small and medium enterprises and self-employment dominate. These are the main buyers for tilapia in the Philippines. In October, a 50% surplus of tilapia was reported by BFAR's Willy Cruz in some farms on Luzon Island. Early in the pandemic in China, some 4-5 million tonnes of carps remained unsold. In Norway, salmon remained resilient with retail prices stabilising. The salmon industry is dominated by large corporations and cannot skip cycles or pull out due to length of each cycle and structure of the business.

Food security became a 'red flag' in many countries. Singapore imports 125,000 tonnes of seafood and aims to have 30% of local production by 2030. Urban aquaculture using recirculation aquaculture systems (RAS) has already been gaining interest but for a bigger country like China, food security is not only about putting food in supermarket shelves. It has stronger implications all the way upstream to include protein meal supply, i.e. the core basics of animal and aquaculture feed.

What lessons can Asia learn? There is no turning back. We need to focus on marketing, innovative routes to market, value adding and sustainability. Asia must ratchet up from a tradition of production focus and aspirations of a volume supplier. With a scenario of lockdowns, closure of borders and working from home, Aqua Culture Asia Pacific will continue to play the important role in the dissemination of knowledge and networking. If anything, we are reminded of our mission statement.

If you have any comments, please email: zuridah@aquaasiapac.com



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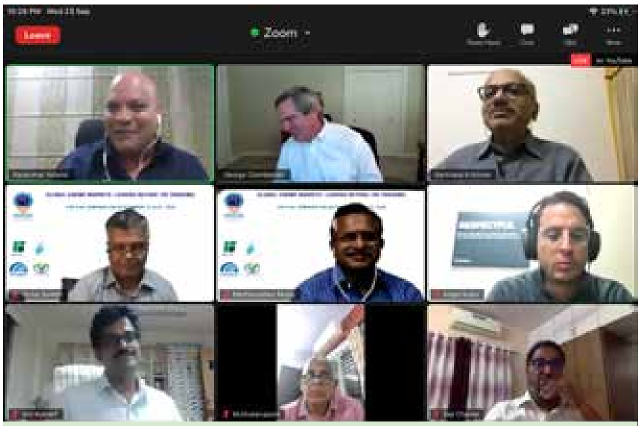
Global shrimp markets: Looking beyond the pandemic

On September 24-25, the Society of Aquaculture Professionals (SAP), India organised a 2-day webinar on “Global Shrimp Markets: Looking Beyond the Pandemic”. In a press release, Dr Arul Victor Suresh, SAP President said, "In March, when we had our first lockdown, while shrimp farmers battled uncertainties in the supply chain and managed diseases, they were concerned with prices and whether there would be buyers for their harvests. There are many forecasts on shrimp demand in major markets. We believe that it is important to combine such information with shrimp supply scenarios directly from major producers in Asia and Ecuador.”

Keynote speaker, George Chamberlain, President, Global Aquaculture Alliance, said, “The global shrimp supply has been on a rising trend for the last few years and at the same time, prices have been falling, indicating that markets were getting saturated in spite of the emergence of China as a major market in recent years.” Citing the case of avocado as an example of what unified marketing efforts by the producers and sellers can achieve, Chamberlain called for a similar effort in which shrimp consumption is promoted.



At the panel discussion on demand and markets on day 1, Dr Arul Victor Suresh, SAP President (middle row, left) led a team of SAP members and speakers. MPEDA’s Dr Anilkumar (last row, left) said that India increased its exports of cooked and breaded products to the US market indicating that Indian processors have the flexibility to meet the market requirements at short notice.

Demand and markets

In his introduction to the session on shrimp demand and markets, moderator, Ravi Yellanki, Vaisakhi Biomarine, said that the industry in India has come to terms with the reality of the Covid -19 pandemic. Industry, hatcheries in particular, have realised the global nature of shrimp farming; it starts with imports of broodstock from the Americas and exports of final products to the US, Europe and China. The pandemic has changed the way hatcheries operate – staff are not allowed to leave the premises, farmers agree for hatcheries to send post larvae directly to laboratories for disease and health checks, and size specifications are checked via videos.

Demand from India’s leading markets has been encouraging; China was the first to recover and demand picked up in April, May and June. The demand from the food service segment was better in the US as compared to that in China.

In his presentation on the “Impacts in the US shrimp markets”, Angel Rubio, Chief Analyst at Urner Barry, said that retail sales of shrimp rose during the pandemic but still could not compensate for the lost sales in the food service segment. Prices of shrimp fell as a result but retail establishments passed the benefit of low prices to the consumers through promotions. It is likely that the experience of cooking shrimp at home will encourage consumers to increase their purchase of shrimp in retail outlets even after the pandemic is over.

With regards to markets in Europe, Willem van der Pijl, who recently started Shrimp Insights, a data service specific to global shrimp trading, said that shrimp consumption in Europe was down by 6% up to June 2020, but the shrimp mostly impacted by this drop was the ocean-caught premium shrimp. Farmed penaeid shrimp were much less affected at only 1%. Due to the opening of restaurants, the summer sales of shrimp are believed to have been healthy. “Although data is not yet available, during July and August, sales increased according to importers and wholesalers in the Netherlands, Belgium and Germany. However, the ongoing second wave of the pandemic points to the possibility of lockdowns during the winter and this would negatively impact consumption leading to less new orders between October and February/March 2021. Vietnam and Latin American suppliers have a competitive position in the European markets requiring other Asian suppliers to reassess their competitiveness. Vietnam supplies more than 70% of the demand while India supplies just 5%,” said van der Pijl.

Vincent Lin of Grobest Seafoods said that China imported 703,000 tonnes of shrimp in 2019 but average prices were sliding downwards. In 2019, the surge in imports was led by Ecuador (321% at 322,000 tonnes); India (338% at 155,000 tonnes and Thailand (58% at 39,000 tonnes). China has maintained an increasing trend of imports until January-February 2020. Demand picked up in June but dropped in July 2020 when fragments of coronavirus genetic materials were found on the packaging of shrimp imported from Ecuador. Consumer confidence was severely impacted and the import volumes and prices tumbled as a result.

China’s shrimp production has been affected due to the emergence of new diseases, so local production will go down and be directed to the premium live shrimp market. Lin added that in the future, imports of some superior grade chilled or frozen head-on shell-on (HOSO) shrimp may enter this market. Resumption of imports at or beyond volumes in 2019 will begin only when consumers gain confidence that frozen food is not a risk factor in the spread of coronavirus. India exports headless shell-on

(HLSO) and there is room for India with good quality raw material for shrimp paste. Chinese consumers appreciate good quality shrimp and are willing to pay for quality.

Pawan Kumar Gunturu of Sprint Foods, India provided a perspective from Indian exporters. The sudden lockdown resulted in difficulties in operating processing plants and forced migrant workers to move back to their home bases. Indian exporters faced cancellations or postponement of purchase orders. Decline in prices and cancellation of export incentives by the government have caused further hardships for the exporters. Gunturu showed that there has been an increase of 5% in value-added products from India. He added that India needs to build on its strengths and move into more value added products in the near future.

During the panel discussion, Dr P. Anilkumar, Marine Products Export Development Authority (MPEDA), indicated that Indian shrimp exports have declined by about 15% but the exports of cooked and breaded products to the US have registered an increase indicating that Indian processors have the flexibility to meet market requirements at short notice. S. Santhana Krishnan of Maritech, a seafood and aquaculture consulting firm, said that India's ability to supply large sized shrimp can be leveraged to serve niche markets globally.

Supply scenarios

Samson Li, Grobest Feeds CEO, and experts from leading shrimp producing countries discussed recent developments in Vietnam, Indonesia, India and Ecuador amidst this pandemic. Li remarked that supplies from Vietnam have not been severely affected due to the significant domestic market, and the sustained demand from the export markets. Furthermore, strict control of the pandemic and sensible lockdown policies may have minimised the disruptions in supply. However, Vietnam may face a contraction in production, from 630,000 tonnes in 2019 to 570,000 tonnes in 2020. For the long term, Li predicted a strong growth and higher productivity through intensification which has been happening over the past two years, including a return to 2019 production levels in 2021 and from then on a 3% annual growth.

"Initially, Indonesia did not follow a strict lockdown," said Haris Muhtadi, CJ Feeds who also described its intensive culture practices. "The first half of 2020 saw a slight production increase and export of shrimp when compared to the same period in 2019. Indonesia's production in recent years reached close to 350,000 tonnes in 2019 and the production in the first six months of 2020 was estimated to be about 200,000 tonnes. The USA remained the largest importer of Indonesian shrimp, buying nearly 65% of production." Haris estimated that there may be a drop in production in the second half of the year due to disease challenges, and Indonesia may end up with a decline of about 6-7% in production by the end of the year.

According to Paresh Kumar Shetty of Avanti Feeds, the sudden and strict early lockdown in India, resulted in many disruptions affecting shrimp production and processing. Lower shrimp prices also dampened farmers' spirits forcing early harvests of small shrimp (size 100-140/kg). While the lockdown has been relaxed, labour availability continues to be an issue. Also, farmers are facing production challenges in many regions. As a result, Shetty said that India's farmed shrimp production is likely to decrease to about 675,000 tonnes in 2020, from about 800,000 tonnes in 2019.

Gabriel Luna, an industry analyst in Ecuador's shrimp business elaborated on the phenomenal growth of shrimp production and exports in the past 10 years; exports grew 4X in 10 years, reaching 630,000 tonnes in 2019. By August 2020, the country had reached about 450,000 tonnes, a 6% year-on-year increase. This increase was despite the difficulties due to the lockdown and exports to China which was Ecuador's largest market in 2019. Farmers were unable to harvest their crops in April and May due to the lockdown, followed by disruptions in exports to China in July. Prices collapsed to historic lows as a result. "Fortunately, we could quickly increase sales to the USA and Europe and presently have achieved a good redistribution of markets. The decline in prices has affected profitability, " said Luna, adding that Ecuador's producers will seek to improve productivity not by expanding land area or intensification but by focusing on improvements in shrimp growth and survival, and profitability by going into niche markets, such as organic shrimp.

Panellists at the end of day 2, noted that shrimp supply from the major producing countries has not been seriously impacted by this pandemic and global supply of farmed shrimp may decline by about 10% in 2020 as compared to 2019. Global trade of shrimp has not been affected to a large extent except in the case of China. However, lower shrimp consumption in the US where the increase in retail sales has not been compensated for the loss of food service sales means that unsold inventories would be fairly high. Further declines can be expected in the winter due to anticipated restrictions in restaurant operations. Shrimp prices have been negatively affected due to the disruptions from the lockdown as well as the loss of consumer confidence in China. While the low prices have been used to stimulate some of the consumption, the response in the major producing countries to low prices will drive future decisions on production, types of products, market focus, and farming technologies. Watch a recording of this conference at the official SAP YouTube link, https://m.youtube.com/channel/UCzfxp-IVy8IFXP16iTvH_Lg

The day 2 panel discussions on supply scenarios from Vietnam, Indonesia, India and Ecuador, noted that shrimp supply may decline by about 10% in 2020 as compared to 2019.

Minh Phu appeals on shrimp anti-dumping duty

Vietnam's Minh Phu Seafood Joint Stock Company said that it would appeal against the decision by the US Customs and Border Protection (CBP) that frozen shrimp products exported by the company to the US market were subject to duties, said vietnamnet.vn. According to CBP Minh Phu violated the regulation on anti-dumping duty levied on Indian shrimp. In October, based on the Enforce and Protect Act, CBP reached this conclusion as Minh Phu did not provide sufficient evidence as required, to prove that the company did not use India's shrimps to export to the US. The consideration for an appeal is expected to last for 60 days from the date it is received. In case the appeal fails to bring the desired results, Minh Phu will continue to appeal to the International Court of Arbitration. From late July 2019, Minh Phu said it had stopped importing shrimp from India, since raw material in Vietnam was sufficient for its production and processing activities.

Kontali acquires Seafood TIP

In October, Kontali Analyse AS (Kontali) completed its acquisition of Seafood TIP (and *Shrimp Tails*). Thomas Aas, Managing Director, Kontali, said that Seafood TIP has a strong foothold and knowledge within the seafood industry and will strengthen Kontali's position in systemising the world of fisheries and aquaculture. With Seafood TIP onboard, the combined analytical capacity and sector coverage is expanded to all major segments within both finfish and crustaceans. The acquisition of Seafood-TIP will not only expand Kontali's geographical presence with its offices in Utrecht but more importantly, it will strengthen Kontali's commitment to increase transparency in the sector through insights and intelligence.

The Seafood TIP team, over the years, has worked tirelessly to become the leading source for seafood market intelligence. With this collaboration, Kontali is confident in expanding the scope and coverage of its intelligence services with Seafood TIP's expertise and knowledge. "We are excited for the growth and development, in the years to come, as Seafood TIP and Kontali continue to systemise global aquaculture and fishery supply chains and help support companies to make increasingly sustainable, profitable and data-driven decisions, " added Aas.

Joint venture to farm sea grapes

Blue Aqua International and Stemcell United Limited (ASX: SCU) will cultivate and farm sea grapes (*Caulerpa lentillifera*) in Singapore on a commercial scale via the jointly incorporated company, SCU Green Aqua Farm Pte Ltd. Stemcell is an Australian mainboard-listed biotechnology and pharmaceutical company focussing on the development, reproduction, culture and extraction of plant stem cells for TCM medicinal, health, beauty and anti-aging applications using its environmentally friendly patented technology. SCU has successfully applied plant stem cell technology on sea grape cultivation at its research base located in the Marine Aquaculture Centre on St John Island, Singapore. It is now moving towards commercial cultivation. The JV combines the strengths of both parties in creating an integrated aquaculture farming system. The collaboration aims to promote the ocean vegetable's unique qualities as a sustainable superfood and plant-based protein.

Philip Gu, SCU CEO/executive chairman commented, "This partnership will further progress our efforts to make a substantial contribution to the Singapore Government's "30 by 30" initiative, which aims to increase Singapore's local food production from its current level of 10% to 30% by 2030." Dr Farshad Shishehchian, Founder, Group President and CEO of Blue Aqua International said, "SCU is an established technology company in the plant-based stem-cell business. This joint incorporation of SCU Green Aqua Farm embodies our continual efforts to build a circular economy in aquaculture, starting with our own production systems towards the development of sustainable nutrition globally."

Thai Union venture fund invests in four new food tech companies

In September, the world's seafood leader Thai Union Group PCL announced four new investments in the food tech space through its recently created venture fund, which focuses on alternative protein, functional nutrition and value chain technology startups. These are Alchemy Foodtech Pte Ltd., a Singapore-based diabetes food tech innovation company; Manna Foods Co, an insect tech and e-commerce company in the US; and HydroNeo GmbH, which develops comprehensive solutions for smart aquaculture management based in Germany and Thailand. All three companies were part of the first cohort of SPACE-F, the first food tech incubator and accelerator program in Thailand, which Thai Union is a founding partner, alongside Mahidol University and Thailand's National Innovation Agency (NIA). In addition, Thai Union will invest in VisVires New Protein, a Singapore-based food tech investment fund, to deepen its network of identifying co-investment and collaborative opportunities in the global agrifood tech ecosystem. Thai Union's venture fund was launched in 2019 with an initial commitment of USD 30 million. It is investing in early-stage entrepreneurial companies that are active in these areas and will actively partner with these companies to support and accelerate their development.

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Culture in concrete tanks: Growth of white shrimp at varying stocking densities

In Batam, Indonesia, juveniles cultured in concrete tanks at a stocking density of 300 PL/m^2 showed the best daily growth after 75 days.

By Romi Novriadi

Concrete tanks of 8 x 8 x 1m sizes were used for the intensive production of Litopenaeus vannamei

Recently, many shrimp farms, especially in Indonesia, have shifted from extensive or semi-intensive pond culture to the intensive, and even super-intensive (or locally known as supra-intensive) technology systems with high stocking density; these range from 110 – 500 post larvae (PL)/m^2 in intensive culture and >500 PL/m^2 for super-intensive culture systems. There are advantages and disadvantages of using this super-intensive technology. According to Samocha (2019), the high stocking density of shrimp in intensive systems will lead to greater yields, which are more efficient in the use of the culture environment. However, high inputs of nutrients and limitations on water exchange will create water quality problems that do not usually occur in traditional or semi-intensive farming systems. If not addressed quickly and correctly, it will lead to the increase in ammonia-nitrogen (NH_3-N) and nitrite-nitrogen (NO_2-N) levels, development of dense microbial communities and outbreaks of shrimp diseases.

The negative correlation between stocking density and growth performance of shrimp has been well established. Most of the trials were conducted using biofloc technology (BFT), zero water discharge-recirculating aquaculture systems and even in the commonly used method in Indonesia in earthen ponds lined with HDPE (high density polyethylene) plastic. The degradation of environmental carrying capacity conditions, especially dissolved oxygen (DO) levels, seems to show a trend of lower individual growth of shrimp. However, as farming technology advances, greater productivity and efficiency with respect to density and water quality conditions can be improved and optimised.

The aim of this study was to investigate the effects of density and water quality conditions on the growth performance of the white shrimp *Litopenaeus vannamei* cultured in concrete tanks.

Experimental details

This study was performed at the Batam Dae Seng Indonesia farm located in Batam, Riau Island province, Indonesia. *L. vannamei* post larvae (PL8 weighing 0.03 - 0.05g) were obtained from PT Suri Tani Pemuka's hatchery in Anyer, West Java. These were acclimated and nursed in an indoor nursery system for 14 days to a size ready for stocking into the tanks. At the start of the production trial, these juvenile shrimp (PL20-22; mean initial weight, 0.3g) were stocked into 32 semi-indoor concrete tanks (8 x 8 x 1 m) at four different densities: 300, 400, 500 and 600 PL/m^2. The production period was 75 days. Tanks were filled with water with a salinity of 30 – 33 ‰. The primary source of mechanical aeration was with an air disc fine bubble diffuser, with one 0.5HP paddlewheel (Minipadd™) providing additional aeration. Water exchange was 5 – 10% throughout the 75 days trial.

Feed management

Shrimp in all the tanks were fed with the same diet (33 – 35% crude protein, 5% crude lipid; Indonesia Evergreen Agriculture, Lampung Selatan) throughout the growth trial. The amount of feed used in this experiment was calculated based on the expected weight gain of 1g/week, a feed conversion ratio (FCR) of 1.4 and a weekly mortality of 3% during the grow-out period. During the trial, shrimp were fed six times per day and the daily ration was adjusted based on the percentage of body weight after sampling the shrimp on a weekly basis.

Growth sampling and water quality

Shrimp were sampled weekly throughout the production cycle using a hand net (0.5m in diameter and 1cm mesh size) to collect approximately 20 – 30 individuals per tank. Water quality (DO, pH, temperature, salinity, total dissolved solids, conductivity and oxidative redox potential) was monitored four times/day (06.00 – 07.00h; 14.00 – 15.00h; 17.00 – 18.00h and 23.00 – 24.00h) using real-time water quality sensors (Aqua Troll 500, In-Situ Inc., USA) and managed by AquaEasy Smart Aquaculture apps (BOSCH, Singapore). Secchi disk readings were recorded once a week (Table 1).

Ammonia nitrogen (NH_3-N) was analysed with ultraviolet/visible spectrophotometer (Lambda XLS, PerkinElmer, USA) once a week. Nitrite nitrogen (NO_2-N) and nitrate nitrogen (NO_3-N) were analysed using HACH DR890 colorimeter (Hach Company, USA) twice a week (Table 2). At the end of the growth trial, shrimp were harvested fully, counted and batched weighed to calculate the final biomass, final weight, percentage weight gain (%WG), FCR, survival (SR), and voluntary feed intake (VFI) as shown in Table 3.

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Statistical analysis

All growth parameters were analysed using one-way analysis of variance (ANOVA) to determine the significant differences among treatments followed by Tukey's multiple comparison tests to determine the difference between treatment means in each trial. All statistical analyses were conducted using SAS system (V9.4. SAS Institute, USA).

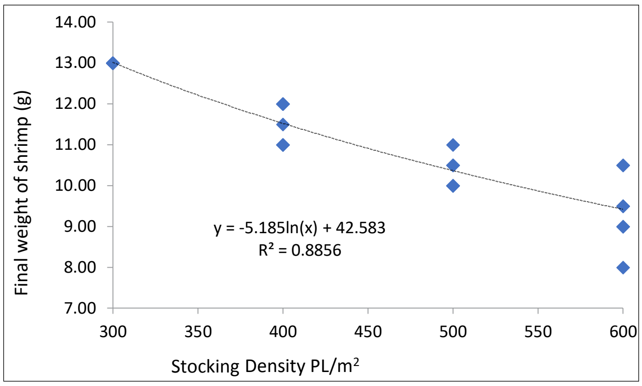

Figure 1. Shrimp growth performance at four stocking densities measured weekly in 32 concrete tanks during the 75-day culture period. Data are means ±SD of 8 replicates.

Growth performance versus density

During the growth trial, the levels of DO, pH, total dissolved solids, oxidative redox potential, salinity and temperature, presented in Table 1 were within the acceptable range to support optimal growth of the shrimp (Boyd and Tucker, 1992). The ammonia levels ranged from 0.22 ± 0.09; 0.21 ± 0.12; 0.30 ± 0.27; and 0.29 ± 0.44 for 300 PL/m^2; 400 PL/m^2; 500 PL/m^2 and 600 PL/m^2 stocking density, respectively. Meanwhile, for NO_2-N and NO_3-N readings, there was an increasing trend in concentrations with higher shrimp density.

The results of this study indicated that *L. vannamei* juveniles grew faster when reared at a lower density. Our study indicated that the ideal growth of 0.17 ± 0.02g/day was obtained at the stocking density of 300 PL/m^2. This is similar with the study from Krummenauer et al. (2011), where the density of 300 shrimp/m^2 exhibited superior zootechnical performance compared to 450 shrimp/m^2 with ADG of 0.13 ± 0.02g/day using super-intensive culture system in 70m^2 tanks lined with HDPE and 1m of water column. Stocking density has a significant effect in determining the carrying capacity of the tank and is crucial for optimising growth performance and health status of the vannamei shrimp during the culture period. During the culture period, there were no incidences of disease.

Interestingly, in terms of biomass, the density of 300 PL/m^2 yielded the lowest quantity compared to higher density treatments. This means that if we are focusing on yield and profitability, we could also apply high density culture during the production period and maintain the carrying capacity, water quality condition and apply the proper feeding management system.

Measuring water quality parameters (DO, pH, temperature, salinity, total dissolved solids, conductivity and oxidative redox potential) 4 times/day using real-time water quality sensors.

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	Treatments			
	300 PL/m²	400 PL/m²	500 PL/m²	600 PL/m²
Dissolved oxygen (mg/L)	4.90 ± 0.14	4.85 ± 0.30	4.74 ± 0.12	4.75 ± 0.15
pH	7.35 ± 0.11	7.25 ± 0.17	7.33 ± 0.20	7.27 ± 0.12
Total dissolved solids (mg/L)	30.18 ± 1.36	31.23 ± 1.31	32.09 ± 1.14	32.17 ± 2.82
Oxidative Redox Potential (mV)	257.41 ± 28.09	263.32 ± 27.82	261.72 ± 33.18	263.05 ± 25.92
Salinity (‰)	32.69 ± 0.64	33.00 ± 0.41	32.95 ± 0.33	32.89 ± 0.74
Temperature (°C)	29.43 ± 0.25	29.28 ± 0.29	29.32 ± 0.25	29.34 ± 0.31

Table 1. Summary of water quality parameters for the four treatments throughout the 75-day culture period. Data was obtained by using sensors and recorded using AquaEasy Smart Aquaculture apps. Values are presented as mean ± SD of 8 replicates.

	Unit	Treatments			
		300 PL/m²	400 PL/m²	500 PL/m²	600 PL/m²
Ammonia	mg/L	0.22 ± 0.09	0.21 ± 0.12	0.30 ± 0.27	0.29 ± 0.44
Nitrite (NO_2-N)	mg/L	0.09 ± 0.12	0.11 ± 0.08	0.14 ± 0.30	0.14 ± 0.31
Nitrate (NO_3-N)	mg/L	42.88 ± 12.47	64.87 ± 20.56	78.55 ± 28.94	80.94 ± 30.22

Table 2. Summary of nitrogen characteristics dissolved in the water for the four treatments throughout the 75-day culture period. Values are presented as mean ± SD of 8 replicates.

Density (PL/m²)	Biomass (kg)	Final weight (g)	Weight gain (g)	ADG (g)	Feed input (kg/tank)	FCR¹	Survival rate (%)
300	133.2 ± 5.1ᵇ	13.0 ± 0.2ᵃ	12.5 ± 0.2ᵃ	0.17 ± 0.02ᵃ	210.00 ± 0.00ᵃ	1.58 ± 0.06	53.36 ± 2.17
400	156.9 ± 15.7ᵃᵇ	11.6 ± 0.5ᵇ	11.2 ± 0.5ᵇ	0.16 ± 0.00ᵇ	220.25 ± 36.30ᵃ	1.40 ± 0.18	52.98 ± 4.72
500	172.8 ± 10.0ᵃᵇ	10.3 ± 0.4ᶜ	10.0 ± 0.4ᶜ	0.15 ± 0.01ᶜ	245.75 ± 27.29ᵃ	1.43 ± 0.20	52.36 ± 2.70
600	198.7 ± 32.0ᵃᵇ	9.3 ± 1.0ᵈ	8.8 ± 0.9ᵈ	0.14 ± 0.02ᵈ	280.88 ± 34.48ᵃᵇ	1.43 ± 0.10	55.94 ± 7.73
P-value	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	0.0850	0.4822
PSE²	19.8827	0.5728	0.5515	0.0139	28.5431	0.1468	4.8442
Linear Regression							
r^2	0.6173	0.8762	0.8849	0.7778	0.4858	0.0988	0.0074
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0798	0.6388
[1] FCR = Feed conversion ratio [2] PSE = Pooled standard error [3] ADG = Average Daily Growth							

Table 3. Growth performance of shrimp (mean initial weight of 0.3 g) fed the experimental diets for 75 days. Values are means ± SD of 8 replicates. Results in the same columns with different superscript letters are significantly different (P<0.05) based on analysis of variance followed by the Tukey's multiple comparison test.

Is EHP now the key health concern for Asian shrimp producers?

While key to its management are strict biosecurity and disease surveillance practices, infection rates in tested ponds have risen with an average infection rate of 84.8% in 2019 and 93.5% throughout the first half of 2020.

By Pau Badia, Nitrada Yamuen, Chatbongkot Meakniti, Kultida Khwankuea, Ratchakorn Wongwaradechkul, Audrey Burkard, Marius Nicolini, Tarinee Limakom, Anton Immink, Olivier Decamp, Ralf Onken and Andy Shinn

Enterocytozoon hepatopenaei (EHP), a fungal microsporidian parasite commonly infects the hepatopancreas of the whiteleg shrimp (*Penaeus vannamei*). It is now arguably the key health concern for many producers across Asia. The ingestion of spores results in the infection of epithelial cells lining the tubules of the hepatopancreas. The subsequent proliferation and release of spores either by exocytosis or consequential cell breakdown, impacts on the digestive capacity of shrimp, resulting in slow growth and a notable variation in the size of stock. Chronic infections can lead to the loss of stock.

Controlling EHP in farm environments is a challenge. The key to its management lies in strict biosecurity and disease surveillance practices, i.e. in the rigorous disease testing of stock, feeds and water at each point in the production chain; avoiding the introduction of infected batches of post larvae and water into ponds and avoiding the overstocking of culture systems. It is also ensuring effective water and waste management and the comprehensive disinfection of ponds between crop cycles. As the original source of EHP has as yet to be determined, it is important that all alien organisms are excluded and their entry into culture systems are prevented. Likewise the on-farm movement of equipment between ponds should be avoided where possible unless robust disinfection procedures are in place. The number of shrimp, the sampling strategy and the diagnostic methods used, are appropriate for the sensitive and confident early detection of infections.

"The most worrying finding from the study was that infection rates have continued to rise, with test results from the last six months of 2019 indicating an average infection rate of 84.8%."

SHRImp

The disease management component of the Shrimp Health Resources Improvement project (SHRImp), a project funded by IDH-the Sustainable Trade Initiative and the Walmart Foundation and managed by the Sustainable Fisheries Partnership (SFP) and ThinkAqua, is conducted by a collaborative team of scientists from Benchmark R&D (Thailand), FAI Farms Limited and INVE Thailand who have set up mobile shrimp health clinics in three Thai provinces to support farmers in their shrimp health testing needs.

The clinics operating out of the shrimp clubs offer health evaluations and molecular disease testing for early mortality syndrome/acute hepatopancreatic necrosis (EMS/AHPND), EHP and white spot disease syndrome (WSSV). Farmers can drop off samples, which are processed while they conduct club business. The results are relayed back to the farmer by phone and through a purpose-designed software platform that runs on a mobile phone app-MyShrimp.farm. Access to the application has several layers of security so that the confidential results can only be seen by the farmer, who can decide who they wish to share their results with.

In 2019, 129 farms, 451 ponds and >13,250 shrimp were tested. Infection rates across the three provinces ranged from 44.1%-95.2% (average was 48.6%). Infection rates in earthen or slope-only lined ponds were higher (65.9%) than fully lined ponds (46.3%). Looking at other combinatorial factors such as the presence of shrimp toilet or not, indicated that infection rates in earthen and slope-only lined ponds with no shrimp toilet were higher (81.1%) when compared to fully lined ponds with a shrimp toilet (60.9%). The most worrying finding from the study was that infection rates have continued to rise, with test results from the last six months of 2019 indicating an average infection rate of 84.8%, while the testing of a further 262 ponds throughout the first half of 2020 suggest an infection rate of 93.5%.

(See EHP Fact Sheet on diagnosis, prevention and management in hatcheries and grow-out ponds on p34-35).

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Modulating shrimp and water microbiota in hatchery and nursery systems

New investigations with molecular techniques can help our understanding of microbial interactions and the impacts of bioremediation and probiotic bacteria in shrimp culture.

By Ana Rodiles, François Cellier, Stephane Ralite, Eric Leclercq and Mathieu Castex

Shrimp hatcheries are crucial to successful harvests and overall, to the aquaculture industry as providers of healthy, robust and predictable fast-growing post larvae (PL). In recent years, nursery systems have been developing globally amidst a number of bottlenecks associated with the stocking of early PL into open pond systems. Nurseries offer a more controlled environment enabling the implementation of stricter biosecurity measures, which minimise the propagation of opportunistic pathogens and the risk of infectious outbreaks at the sensitive PL stage. Both culture phases typically use closed systems with various water renewal rates.

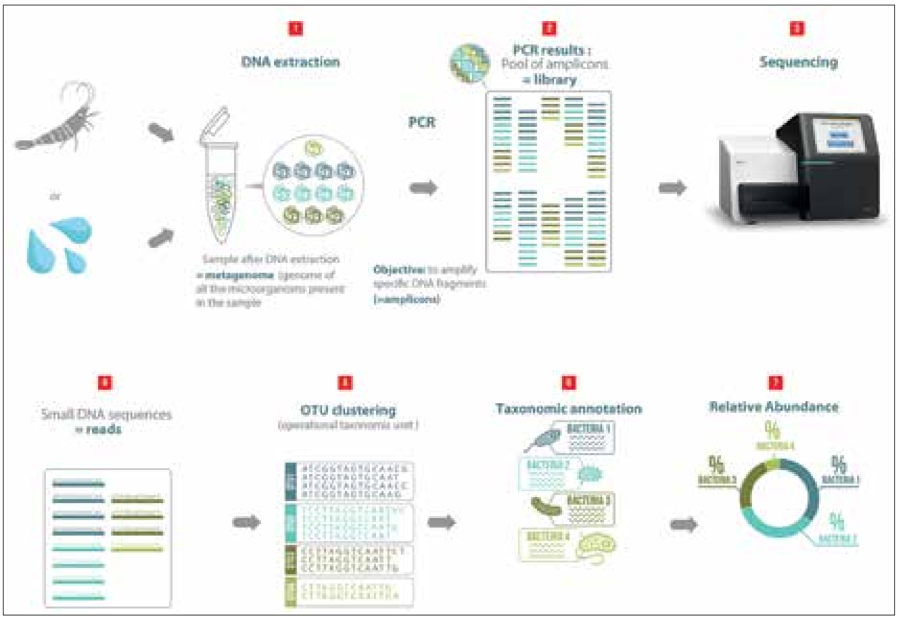
In such systems, pathogenic bacteria may bloom, and organic matter may accumulate, resulting in toxic inorganic nitrogen build up. Additions of new water, effectively diluting any unwanted elements, can be a solution but risk introducing pathogens, and often cause abrupt environmental fluctuations. Such fluctuations may lead to imbalances in the water and intestinal microbiota, by stressing the closed system and hence the shrimp, which can become more vulnerable to outbreaks of opportunistic pathogens. In an attempt to control bacterial pathogens, broad-spectrum antibiotics (ABX) are sometimes applied indiscriminately. This brings in the risk of exacerbating a disequilibrium of the microbiota while promoting antibiotic resistance and the risk of antibiotic contamination in harvested shrimp.

At Lallemand Animal Nutrition, we support hatchery and nursery operations with dedicated microbial solutions for bioremediation, biocontrol and in-feed probiotics. The impacts of such microbial inputs are often difficult to appreciate due to the complexity of the microbial interactions at play and the analytical methods needed to decipher them. This article gives an example of how new technologies can help our understanding of the microbial interactions and the impacts of bioremediation and probiotic bacteria in shrimp culture.

Technology to understand microbiota

While classical microbial plating systematically selects a portion of the bacterial community depending on the growth medium used, molecular techniques give access to the whole microbial community (Figure 1). The substrates such as faeces, intestinal mucosa, culture water, pond soil or tank biofilm are first sampled. The DNA or genome of the whole bacterial community in the sample is first extracted with a dedicated DNA extraction kit. Then DNA from a specific region is multiplied by PCR and the resulting amplicons (DNA fragments) are sequenced using an amplicon sequencing machine. This results in millions of sequenced DNA fragments that are, using bioinformatics tools, sorted out and matched against a worldwide database of microbial DNA. The output is a complete profile of the bacterial species and their relative abundance in the sample, which includes non-cultivable bacteria, making it possible to approach the real composition and diversity of the microbiota population.

Lallemand Centres of Excellence have established partnerships with research entities around the world and satellite collaborations to fast forward the development and innovation of microbial solutions supporting sustainable animal production, and continuously document the biology, mode of action and benefits of existing yeast and bacteria strains dedicated to animal nutrition. These centres have internalised the amplicon sequencing process to develop a harmonised approach to analyse complex microbial community samples.



"... the input of beneficial bacteria, directly in the water or in the feed, can be tracked back and be found in significantly higher numbers in the rearing water and within the animals."

Figure 1. Amplicon sequencing workflow: extracting samples DNA, PCR, amplicon sequencing and bioinformatics analysis.

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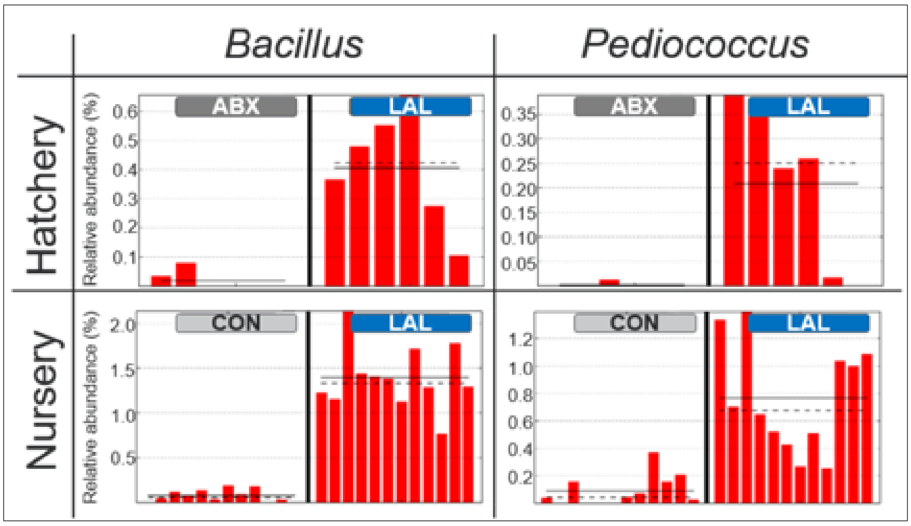


Figure 2. Relative abundance of *Bacillus* and *Pediococcus* in ABX and LAL treatments in hatchery water and nursery shrimp gut microbiota ($P<0.05$). Individual data, average (straight line) and median (dotted line) for each treatment are shown.

Application of amplicon sequencing tools in shrimp culture – practical examples

We carried out a controlled experiment in Vietnam covering both the hatchery and nursery phases in the culture of whiteleg shrimp (*Litopenaeus vannamei*). The hatchery phase was performed in a commercial hatchery (6,500L tanks, 200 nauplii/L, 31°C, 32ppt, no water exchange) and compared different Lallemand products (LAL) to antibiotics (ABX) applied in water under routine husbandry practices. Shrimp were then transferred to an adjacent pilot-scale nursery (250L tanks, 2 PL10/L, 20ppt, 28°C, 15% water exchange/day) where LAL and competitor products (COMP) were applied against a negative control under standard management. The trial assessed the changes in gut and water microbiota generated by the treatments and the consequences on performances with the aim to answer practical and frequently asked questions (Table 1).

	Bioremediation products	In-feed probiotic
Hatchery (22 days, n = 6/treatment)		
Lallemand (LAL)	Lalsea PL Pack	Bactocell
Antibiotic (ABX)	OTC (5ppm/day)	-
Nursery (28 days, n = 4/treatment)		
Lallemand (LAL)	Lalsea PL Pack	Lalpack Probio
Competitor (COMP)	Competitor product	Competitor product
Control (CON)	No product	No product
Bactocell: *Pediococcus acidilactici CNCM I-4622 (MA 18/5M);* Lalsea PL Pack: *Bacillus* spp. and Bactocell; Lalpack Probio: Bactocell and *Saccharomyces cerevisiae* var. *boulardii* (CNCM I); OTC: oxytetracycline		

Table 1. Treatments of water bioremediation and in-feed probiotics in a commercial hatchery and nursery under routine husbandry practices.

How can we track the added beneficial bacteria in the water and in the shrimp?

The bacteria within Lalsea PL Pack (*Bacillus* and *Pediococcus*) were tracked back to the genus level. In the water, *Pediococcus* represented 0.2% of the microbiota in the LAL treatment group compared to 0.01% in the ABX treatment group, while *Bacillus* made up 0.4% and 0.02% of the microbiota in the LAL and ABX treatment groups, respectively (Figure 2).

Similarly, in the shrimp gut, the application of LAL products increased by more than 200% the presence of *Bacillus* and *Pediococcus* compared to the control treatment at the nursery phase.

Together, this unequivocally showed that the input of beneficial bacteria, directly in the water or in the feed, can be tracked back and be found in significantly higher numbers in the rearing water and within the animals.

Do probiotic and bioremediation products reduce the prevalence of undesirable bacteria?

Yes. Some undesirable bacteria could not be detected (0% prevalence) in the hatchery water when using LAL products including a family of *Vibrio* species Pseudoalteromonadaceae, (ABX: 1.1%), Thiohalorhabdales (ABX: 0.6%) involved in the tail fan necrosis of the lobster and the genus *Algoriphagus* (ABX: 6.4%) linked with the early mortality syndrome/acute hepatopancreatic necrosis disease (EMS/AHPND) from *Vibrio* through horizontal gene transfer. Furthermore, in the shrimp gut, LAL decreased Enterobacteriaceae family (0.1% *vs* 3.8% in ABX) and bacterial genus from typical aquaculture pathogens such as *Tenacibaculum* (0.03% *vs* 0.4%) and *Nautella* (0.6% *vs* 2.4%).

In the nursery, applying LAL products significantly reduced the prevalence of potential pathogens in shrimp gut from 7.5% to 0.28% (minimum 87% reduction), including *Vibrio* (0.1% *vs* 1.4% in the control), *Acinetobacter* (0.03% *vs* 4%), *Thalassomonas* (0.02% *vs* 0.6%), *Owenweeksia* and *Sedimenicola* (0.02 *vs* 0.2%). With LAL, the Enterobacteriaceae family was reduced (0.1% *vs* 0.3% in the control), no *Cellvibrio* (0.6% in the control) which hydrolyses chitin, and no *Coccinistipes* (0.13% in the control) were detected. This last genus is typically enriched by some microalgal bloom and generally found in locations relatively rich in organic carbon.

“Together, these findings support existing knowledge on the capacity of specific microbial solutions, both in-feed and in water, to reinforce the presence of beneficial microbes while helping to keep potential pathogens at bay”

Can we detect the effect of probiotic and bioremediation products on the presence of beneficial bacteria?

Yes. Figure 3 shows that at the hatchery phase LAL treatment increased *Thalassobious* (40% *vs* 7% in ABX) from the Rhodobacteriaceae family in the gut of all shrimp assessed. This family has been recently suggested as a source of probiotic as it is able to synthesise vitamin B12 and tropodithietic acid (antagonistic to *Vibrio* species) while having a large potential contribution to feed digestibility. Also, in the LAL treatment group, a higher proportion of *Psychroserpens* (2% *vs* 0.2% in ABX) was found in line with previous findings after probiotic yeast feeding.

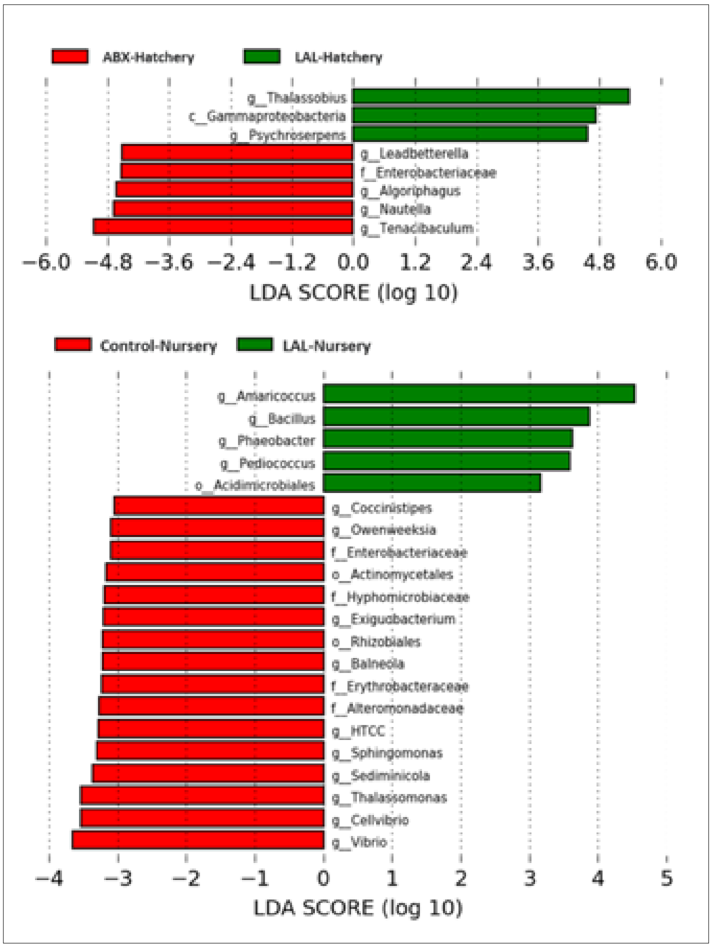


Figure 3. Linear discriminant analysis effect size (LEfSe) of the gut microbiota in shrimp of ABX in hatchery and control in nursery compared with LAL treatment (*P*<0.05).

In the nursery phase, the application of LAL products influenced the gut microbiota compared to the control group with an increase in *Phaeobacter* (1.5% *vs* 0.6%), the genus affiliated to the Rhodobacteriaceae family and proposed as probiotic for its involvement in the biosynthesis of natural antimicrobial compounds and nitrate reduction (Figure 3). Another genus of the Rhodobacteriaceae family, *Amaricoccus*, was also found more prevalent with LAL (14% *vs* 6%). This genus is remarkable for its capacity to store large amounts of poly β-hydroxybutyrate and produces high levels of alkaline phosphatase, an enzyme with health benefits promoting microvilli activity. In the water, *Bacillus* were detected only with LAL (0.04%), together with a significantly higher proportion of *Sphingomonas* (27% *vs* 5%; involved in denitrification) as well as *Rubrivivax* (7% *vs* 1%; carrying nitrogen and carbon fixation) and *Kaistobacter* (8% *vs* 2%; capacity to degrade toxic aromatic compounds).

A positive microbiota has diverse benefits such as increased immune capacity and animal robustness, as well as improved nutritional performance and water quality, while a lower pathogen pressure reduces the likelihood of disease outbreak. Together, these findings support existing knowledge on the capacity of specific microbial solutions, both in-feed and in the water, to reinforce the presence of beneficial microbes while helping to keep potential pathogens at bay.

Does water microbiome impact all life-stages of shrimp similarly?

No. The gut and water microbial population were very similar at the hatchery but showed much more disparities at the nursery stage (Figure 4). The general consensus is that early immature life stage initially acquires its gut microbiota upon mouth opening and first feeding. This suggests that, by positively modulating the water as well as live-feed microbiome via probiotic enrichment, farmers can readily guide and secure the animal's microbiota.

At later stages, gut and water microbiota progressively differentiate as the gut microbiota becomes much more readily influenced by the feed and by its host, the shrimp. This does not hinder the use of water bioremediation and biocontrol bacteria after larval rearing but highlights the need for in-feed probiotic to act on the intestinal microbiota.

Do all probiotic and bioremediation products act in the same way?

No. The detection of the competitor probiotic (COMP; containing other strains of *Pediococcus acidilactici* and *Bacillus* spp.) in the shrimp gut was much lower compared to the LAL treatment group (0.8% *P. acidilactici vs* 0.01%). Interestingly the levels of *P. acidilactici* in the competitor group were also significantly lower compared to the control, highlighting the need to select the right strain and define the right dosage to truly influence the microbial communities.

The LAL treatment group also showed a greater increase in gut symbionts when compared with the COMP group. It was 32% *vs* 7% for the genus *Ruegeria* of Roseobacter, which is considered beneficial. It was 20% *vs* 4% of *Paracoccus*, a natural producer of carotenoids such as astaxanthin; 1.5% *vs* 0.5% of the probiotic *Phaeobacter* and 0.6 *vs* 0.1% of *Erythrobacter* which produces Bacteriochlorophyll-A and contains large amounts of carotenoids (Figure 5).

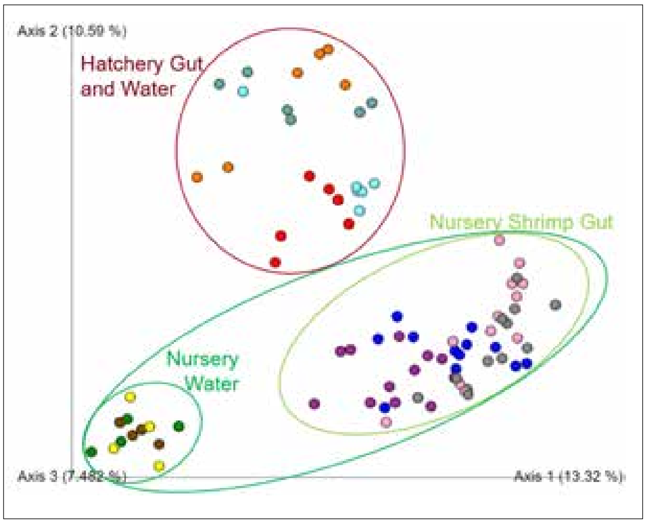


Figure 4. Similarity in microbiota composition based on the presence of bacteria (unweighted unifrac distances beta diversity) in the shrimp gut and rearing water of both trials.

Treatments with LAL products performed significantly better than COMP treatments at reducing the relative abundance of undesirable bacteria (0.9% vs 9%). These include the family Enterobacteriaceae (0.01% *vs* 2%); *Klebsiella* (0.01% *vs* 0.2%); *Enterobacter* (0% *vs* 0.3%) which is associated with drug-resistance and food-borne infections; *Anaerospora* (0.2% *vs* 4%); *Halomonas* (0.1% vs 0.3%) which is related to human and animal pathogens; Legionellales (0.6% *vs* 1.4%) which are intracellular parasites; Thiohalorhadales (0.02% *vs* 0.2%) and *Acinetobacter* (0.02% *vs* 0.3%). With the LAL treatment group, we also showed undetectable levels of opportunistic pathogens such as Nocardioidaceae and *Shinella* that can appear in the gut of diseased shrimp and were present at 0.1% in the COMP group.

These results demonstrate that two apparently similar products can show very different effects even when applied under the same conditions. This emphasises the importance of strain specificity in bacterial products. As a result of improved water and intestinal microbiota, end-point body weight, feed conversion ratio (FCR) and survival improved in the LAL treatment group compared to the COMP group in the nursery. The former group yielded better and more consistent performance (Figure 6).

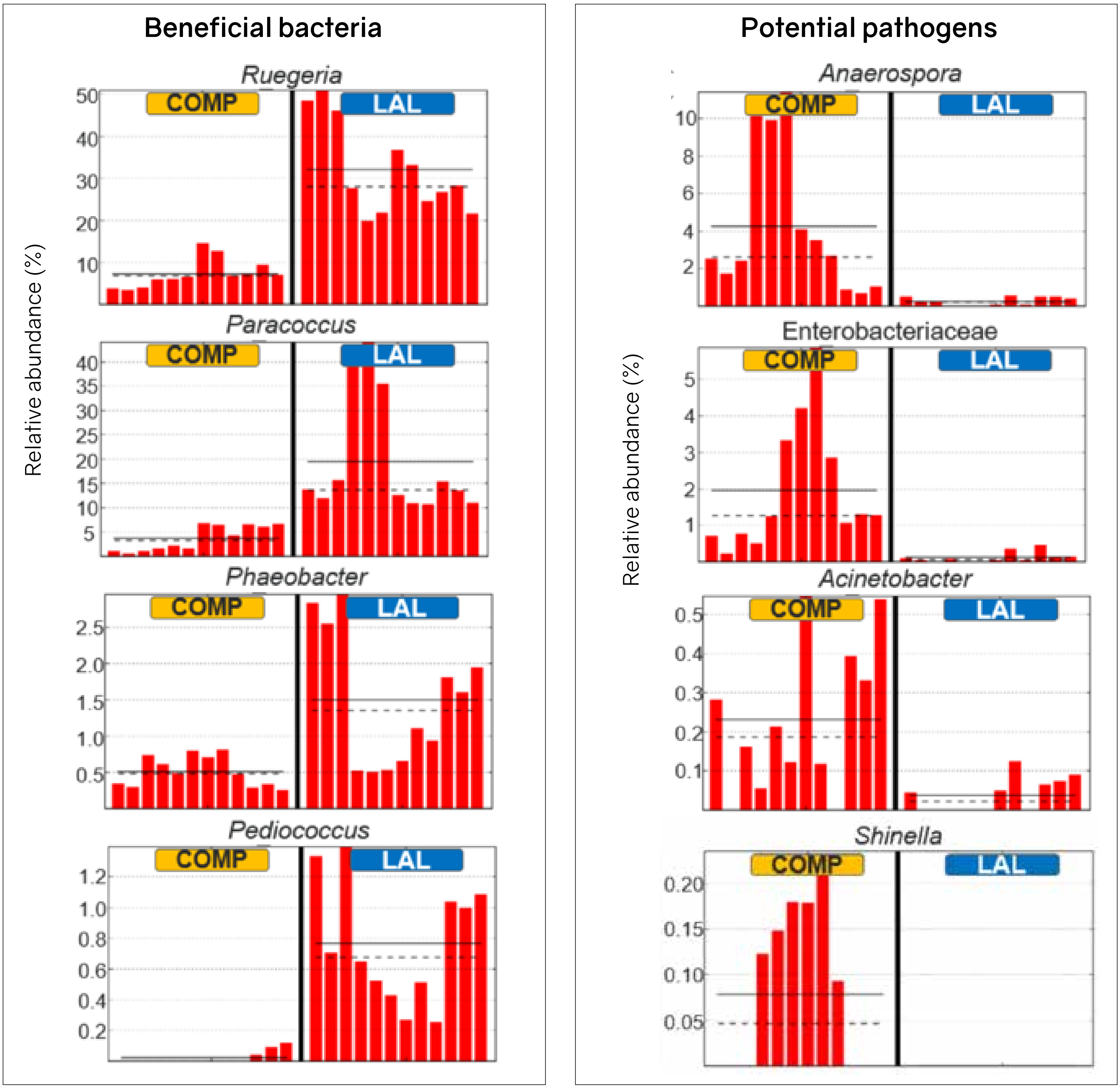


Figure 5. Relative abundance of beneficial bacteria and potential pathogens in the nursery shrimp gut microbiota of COMP and LAL treatments (*P*<0.05). Individual data, average (straight line) and median (dotted line) for each treatment are represented.

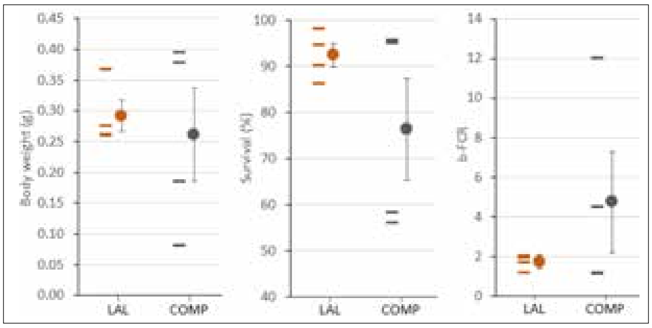


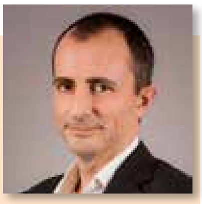
Figure 6. Nursery stage performance of COMP and LAL treatments. Lines represent individual tank replicates; dot and vertical bars, the average ± SE of body weight, survival and b-FCR for each treatment.

Conclusion

Latest molecular tools shed some light in the understanding of microbiota and on how microbial products and process work. This trial demonstrates that we can track the products in the water and in the shrimp. Lallemand microbial solutions reduce undesirable bacteria pressure and increase symbionts with a particular effect on the family Rhodobacteriaceae, including members of the beneficial Roseobacter group (*Phaeobacter* and *Ruegeria*), *Paracoccus* and *Thalassobious*.

It is particularly important to positively modulate water microbiome in the hatchery, as it is tightly linked to shrimp gut microbiota at the early stages. The choice of a product containing carefully selected and well documented bacterial strains is crucial to deliver the expected impacts– to support a stable and balanced microbiome towards a healthy growth under real-time conditions.





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Magnetism to enhance *Artemia* quality

A technology which helps hatcheries to become more efficient in producing high quality *Artemia* nauplii in a more sustainable and eco-friendly way without any toxic chemical waste.

Artemia –a keystone species in aquaculture

The genus *Artemia* has been attributed as a keystone species which contributes to the growth of aquaculture globally. Its ability to produce dormant eggs – cysts, that hatch into live nauplii on demand makes *Artemia* a convenient live feed for cultured marine fish and crustacean larvae. Hatched nauplii of *Artemia* are a rich source of protein, lipids and carbohydrates; fish and shrimp larvae can easily consume the suitably sized nauplii which also stimulate the feeding response of the cultured species (Leger et al., 1986). In addition, capitalising on the non-selective filter feeding behaviour of *Artemia*, it is possible to conveniently manipulate the biochemical composition of live nauplii. The filter feeding *Artemia* can rapidly bioaccumulate nutrients deficient in its own nutritional profile; the enriched *Artemia* nauplii are then able to enhance the larval development of marine fishes and crustaceans (Sorgeloos et al., 2001). The increased usage of *Artemia* as live food for larval organisms since the 1980s has enabled aquaculture production to grow beyond that of wild-caught fisheries in the last decade (FAO, 2016).

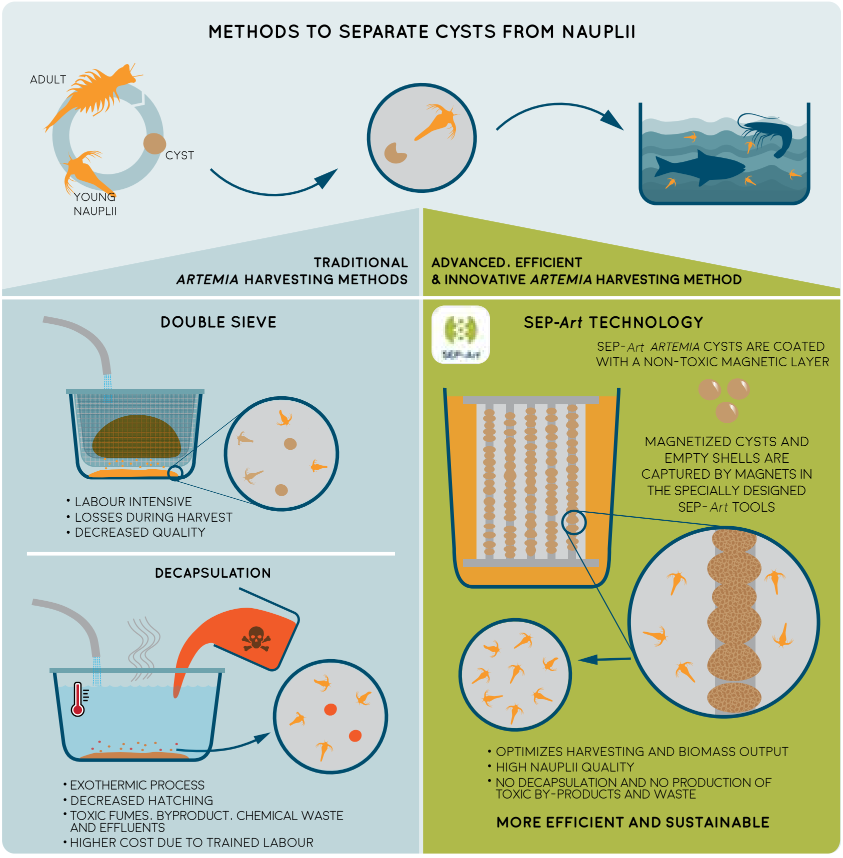

Figure 1. Methods to separate *Artemia* cysts from nauplii.

It is not simply hatching *Artemia*

The process of separating *Artemia* nauplii from cysts remains a significant bottleneck in the production of high-quality live feed. Double sieving, for instance, is still commonly practised, but the method is labour-intensive, inefficient and often results in *Artemia* of poor quality (Figure 1). The method rests on the idea that nauplii will pass through the mesh of a submerged net while the cysts and empty shells are caught in the net. However, those smaller than the mesh size also pass through the net and remain among the live nauplii. During the process, the nauplii – forced to pass through the mesh of the sieve – often suffer physical abrasions. Moreover, nets clog easily, risking increased damage to the *Artemia* nauplii. Dead or damaged nauplii are less attractive prey items; together with the unwanted debris, they increase the bacterial activities at the bottom of the tank. On top of that cysts can block the still developing digestive system of fish and crustacean larvae.

Chemical decapsulation was introduced to overcome the shortcomings of double-sieving (Figure 1). An alkaline hypochlorite solution is used to dissolve and oxidise the hard, brown outermost layer of cysts (i.e. chorion) to free viable embryos ready to hatch. The challenges arising from decapsulation, however, are manifold: the chemical process is complex and requires a highly trained and skilled workforce to attain optimal hatching.

During the exothermic process, heat that may damage or reduce the viability of the embryos is produced. Consequently, it is necessary to monitor and control the oxidation process closely and to interrupt the process at the right time. The need for chemicals and skilled labour makes decapsulation a more costly solution. Lastly, decapsulation produces toxic by-products not easily disposable due to their adverse effects on the environment.

Consequently, double-sieving and/or decapsulation, no longer represent efficient and sustainable methods to produce live *Artemia* nauplii. A better alternative is crucial to allow the aquaculture sector to grow further.

Traditions aside, innovative technology an imperative?

Production by the aquaculture industry has surpassed that of wild-caught seafood and beef and is gaining importance as the main source of animal protein in the future (FAO, 2016). As aquaculture production is expected to increase even further, more efficient and sustainable *Artemia* nauplii harvesting techniques than the traditional methods are crucial. INVE has worked tirelessly for more than 35 years to develop innovative technologies to revolutionise the aquafeed industry. This includes the patented SEP-*Art* technology, which efficiently separates the *Artemia* nauplii from the unhatched cysts. This technology uses magnet-sensitive SEP-*Art* cysts and a SEP-*Art* separation tool that utilises magnets to separate the nauplii from the cysts and empty shells (Figure 1).

It is important to note that SEP-*Art Artemia* cysts are not derived from a specific strain of *Artemia*. These cysts are produced from a patented technology, especially developed to coat the cysts with a non-toxic layer of magnetic material with no effect on the overall hatching characteristics of the cysts. This means that the optimal hatching conditions have not been changed and the same protocols can be followed to hatch the product. In this way, the collection of the nauplii has been simplified and is more efficient and straightforward.

Next-generation tools

During the last 10 years, the SEP-*Art* tools have been continuously improved from initial designs to three next-generation tools: SEP-*Art* HandyMag, SEP-*Art* Cystm2.0 and SEP-*Art* AutoMag. Although the three SEP-*Art* tools differ in their degree of automation, integrated cleaning system and ease of usage, each employs the same concept of using magnetism to separate *Artemia* from the unwanted debris. The process collects the cysts on the submerged magnets for their easy removal from the *Artemia* nauplii suspension. Doing this, the suspension, will turn from a murky brown into a bright orange coloured suspension, only containing *Artemia* nauplii at the end of the process.

In contrast with double-sieving and decapsulation, SEP-*Art* technology maximises the recovery of the hatching output, leading to a 12% increase in biomass (Figure 2). Furthermore, the technology enhances the vitality of the *Artemia* nauplii since no physical force or chemical reaction is employed during the process. Simultaneously, the easy-to-use tools speed up the process of handling and harvesting, allowing even large volumes of nauplii to be handled efficiently. The tools are intuitive to use, making the technology accessible even to untrained workers. This means, using this technology farmers will be able to harvest more and better quality *Artemia* nauplii in a shorter amount of time. Importantly, no chemical waste is produced making it safer for both the user and environment.

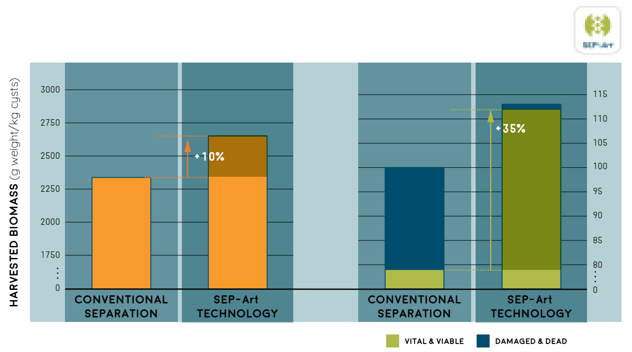

Figure 2. A comparison of the quantity (i.e. biomass) and quality (i.e. survival) of *Artemia* harvested from traditional versus SEP-*Art* methodology.

Live feed of the future

All in all, by employing simple physical principles INVE has developed an innovative technology in the aquaculture live feed sector; this technology provides an eco-friendly alternative to traditional, ineffective, complex and even harmful *Artemia* harvesting techniques. The SEP-*Art* technology is the quintessence of a forward-thinking aquaculture sector ready to feed the world on sustainably reared seafood.

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Channel catfish farming in China: An introduction to common infectious diseases

While production is on the rise, fungal and bacterial diseases threaten channel catfish farming.

By K.P. Sun

The channel catfish *Ictalurus punctatus* was first introduced to China from the US, by the Fisheries Research Institute of Hubei province in 1984. Artificial reproduction was successful in 1987 and brought about the rapid rise in its culture in Hubei, Guangdong, Guangxi, Jiangsu, Liaoning, Jiangxi and other provinces. During the earlier phase of its development (1984–1997), it was mainly farmed in ponds. Later, during the years 1998–2003, cage culture was initiated. The period of rapid development was from 2004 to 2009. By 2013, production rose to a historical high of 247,399 tonnes (Li et al., 2018). In 2018, channel catfish production reached 230,442 tonnes (FishStatJ, 2020).

Today, the main culture systems are mono- or polyculture in ponds, and cages located in rivers, lakes and reservoirs. It is also cultured in high density flow-through systems. One challenge encountered in its culture is disease outbreaks, attributed to the absence of standard culture technology, high density farming systems, poor culture environments, and the pursuit of high yields. Stocking density is usually 128–160 fish/ha and 100–120 fish/m^2 in cages (Li et al., 2018). In this article, we describe some fungal and bacterial diseases affecting the channel catfish in China and suggest some treatment protocols.

Saprolegniasis

This is caused by the following fungal species: *Saprolegnia parasitica*, *Saprolegnia ferax* and *Saprolegnia monica*.

Invasive infections tend to occur during periods with low water temperatures and are often a result of trauma to gills and body surface caused by negligence in operations or transportation. The infection may also develop due to poor water quality or precipitating factors, such as parasites, bacteria or latent virus infections. It is also common in high density farming when water flow is too fast. Saprolegniasis is prevalent in periods with low water temperature (below 18°C) which weaken the fish's immunity and increase mould activity.

At early stages, infected fish are not easily identified with the naked eye. When the infection is thus visible, the hyphae will have grown into the fish's muscle and spread. Small or large cotton-like greyish-white mycelium will grow radially on any part of the body, and spread acutely on the body surface, leading to muscle rot and easily causing bacterial infection (Figure 1).

Prevention and treatment

We recommend immersion in 3–4% saline for 5 minutes after transportation. Disinfection with 30 ppm formalin for 18–24 hours provides an effective alternative. These procedures are permitted in the US, and thus do not hinder exports to that country.

Alternatively, the farmer should enhance farm hygiene, management of net cages, avoid high density farming and unnecessary transportation and stress. During the change in seasons, pay attention to feeding and provide a nutritionally balanced diet; Grobest animal health products and multivitamins should be added before removing the fish from ponds and during transportation to enhance the immunity of fish.

It is also suggested that when removing the fish from ponds and during transportation, utmost care should be exercised so as not to injure the fish. New net cages should be disinfected before stocking fish to prevent wounds from mould infections.

Figure 1. In Saprolegniasis, small or large cotton-like greyish-white mycelium will grow radially on any part of the body.

Skin rot, tail and gill rot disease

The signs and symptoms are many for this disease caused by the bacteria *Flavobacterium columnare* and *Flavobacterium branchiophilum*. There are greyish-white spots on the head, gills and fins, as well as on the body surface of the diseased fish, epidermis hyperemia of the inside of the operculum, eroded gill filaments and pale caudal peduncle. Other symptoms include the disappearance of mucus on the body surface, red and swollen muscles, followed by caudal fin defects, caudal peduncle muscle ulceration and loss. In more serious cases, the tail bone is exposed.

This disease tends to occur when the water temperature is 15–32°C and does not occur under 15°C. Strains with strong pathogenicity invade the gills, resulting in dark yellow spotty colonies on gill filaments and gill hyperemia, which look dark red, with small petechiae, an increase in mucus secretion, and damaged soft tissues that dissolve and fall off, causing severe damage. The gills appear broom-like, and this condition is referred to as gill rot. For strains with weak pathogenicity, the infected sites are the skin and nearby fins; necrosis, discolouration and scale loss at infected sites. Such strains invade deep muscles and result in some skin ulceration. This condition is referred to as tail rot (Figure 2).

Prevention and treatment

Firstly, we suggest an enhancement of farm hygiene management of net cages and to avoid high density farming and unnecessary transportation and stress. During the change in seasons, pay attention to feeding and provide a balanced diet. Our recommendation is to use Grobest animal health product and multivitamins in feed before harvesting the fish from ponds and during transportation to enhance the immunity of fish.

Secondly, during harvesting of fish from ponds and during transportation, avoid injuring the fish and disinfect a new net cage before stocking fish.

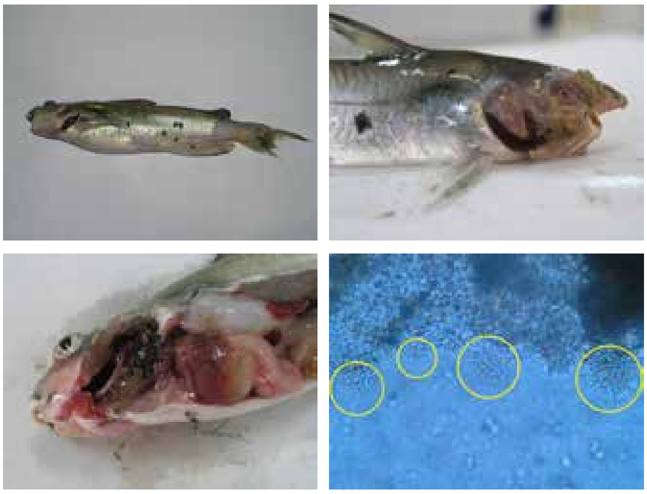

Figure 2. Skin rot, tail rot and gill rot diseases caused by *Flavobacterium* spp. Symptoms include greyish-white spots on the body surface of infected fish (top left), eroded gill filaments (top right) and epidermis hyperemia of the inside of the operculum (bottom left). In microscopic analysis, bacterial colonies appear as small particles grouped together (bottom right).

Enteritis

The signs of enteritis caused by *Aeromonas* spp. in infected fish are abdominal swelling, white gill filaments, fin ray hyperemia—particularly on the dorsal fin, and visible hyperemia spots on the body surface. Infected fish have decreased appetite, thin bodies and mainly swim near the surface of the water. When fish is near death, the head faces upwards and the tail downwards, and it struggles to swim at the surface of the water. On dissection, we observed that the intestinal wall of the fish has mild or severe hyperemia, containing light yellowish-green mucus and a thick discharge when the abdomen is gently pressed. In serious cases, there is water in the gastrointestinal tract, causing the abdomen to swell (Figure 3).

Prevention and treatment

We suggest adding health products normally to enhance the metabolism of the digestion system and effectively promote the formation of a good microorganism environment in the intestinal tract of fish, reduce the content of putrefactive metabolites, enhance the immune functions in the fish's body, and greatly reduce the incidence of enteritis to achieve optimal immunity.

Fish displaying signs and symptoms of diseases in net cages should be treated orally, with medicinal baths, or with both methods as soon as possible according to drug sensitivity test results and management measures on the use of drugs.

Figure 3. Signs and symptoms of enteritis caused by *Aeromonas* spp. are a swollen abdomen (top left); fin ray hyperemia, particularly on the dorsal fin and visible hyperemia spots on the body surface (top right), and the intestinal wall of the fish show mild to severe hyperemia (bottom left).

Catfish edwardsiellosis

This is caused by the bacteria *Edwardsiella ictaluri*. The signs and symptoms differ when the route of infection is through the intestinal tract, it causes sepsis and where it is through the olfactory nerve of the head to the brain, it causes neurological symptoms. The infected catfish can be found floating and swimming at the surface of the water and often suddenly swimming in spirals. There will be bleeding of the mouth, the sides of the body, the abdomen and the bottom of the fin base. The eyes protrude, and ulceration of the forehead (called hole-in-the-head lesion) is common in adult fish (Figure 4). A common symptom is the fish floating vertically in the water with the head facing upwards.

Other signs are abdominal swelling with ascites (containing blood or plasma), pale gills, small skin ulcers on the sides of the body, enlarged liver, kidney and spleen and petechial lesions in the viscera, abdominal cavity and muscle.

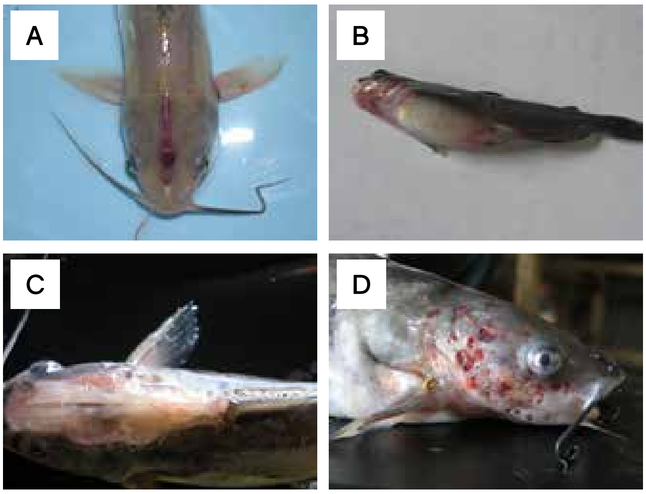

Figure 4. Catfish edwardsiellosis is caused by *Edwardsiella ictaluri*. Signs of this disease are: A. ulceration of the forehead in adult fish; B. abdominal swelling with ascites which contain blood or plasma; C. ulceration and protruding and opaque eyes and D. protruding eyes, small skin ulcers and bleeding.

Prevention and treatment

The pond water should be exchanged regularly and a normal water level should be maintained to prevent water ageing and sudden changes in water temperature. When the disease is suspected, pathogenic bacteria isolation and identification and drug sensitivity tests should be carried out as soon as possible and sensitive drugs should be selected to carry out treatment.

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Recovery from bacillary necrosis of pangasius using a nutrition complex

Supplemented in feed, catfish fingerlings showed liver and kidney recovery after *Edwardsiella ictaluri* bacterial infection.

By Phuong Do, Oanh Dang, Phuong Dang, Audric Touchet, Gaetan Gutter, Philippe Mahl and Hoang Phan

Transfer of juvenile catfish.

Striped or tra catfish *Pangasianodon hypophthalmus* is cultured intensively in Vietnam. In 2019, pangasius production, covering around 6,600ha reached 1.42 million tonnes, and was valued at approximately USD645 million (VASEP, 2019). Culture density is high, commonly 20–40 fish/m^2 during the grow-out stage; fish are harvested when they reach sizes of 800g–1kg/fish. The biomass could reach 40kg/m^2 (Nguyen, 2013).

Bacillary necrosis of pangasius (BNP), a bacterial infection caused by *Edwardsiella ictaluri* is a threat to farms. The disease which is difficult to recognise at the early infection stage was first identified in 2002 (Crumlish et al., 2002). Although striped catfish can be cultured at high densities, its susceptibility to this pathogen increases the chance of spreading the disease quickly among individuals. A late detection of a BNP infection may lead to serious losses of up to 90% mortality (Dung et al., 2004). This disease causes internal damages such as white necrotic or pyogranulomatous foci in liver and kidney (Ferguson et al., 2001) which could not be visibly observed during the early infection stage, thus posing a challenge to disease detection.

Both prevention and treatment are necessary as the disease may occur at any culture stage. Infected fish have eating and digestion problems leading to a reduction in growth rate and meat quality, the latter due to the damage in the internal organs. Liver and kidney of fish, the main organs affected by BNP, play an important role in osmoregulation, digestion and immune system. Fast recovery of these organs after infection could be crucial for fish quality at harvest; however, few studies on recovery of striped catfish after BNP infection have been conducted.

Functional amino acids

In recent years, aquaculture experts have developed an interest in functional amino acids due to their possible roles in body metabolism and homeostasis in fish. Supplementation of an appropriate amount of functional amino acids can assist fish in healing and avoid apoptosis as well as internal stress (Anderson et al., 2016). Use of medicinal herbal plant extracts in aquaculture has gained significant results as chemical agents in terms of animal health and disease control (Gabriel et al., 2019). Artichoke is a popular herbaceous plant used worldwide for its action on dyspeptic and hepatic disorders. In previous *in vivo* assays, its leaf extract showed an improvement in liver tissue structure and hepatic metabolism in animals with hepatocellular carcinoma (Metwally et al., 2011); this extract introduces a potential application against fish liver damage caused by BNP. Another plant that showed a promising recovery effect on kidney injuries belongs to the genus *Orthosiphon*. According to Gao et al. (2015), metabolites extracted from *Orthosiphon* improved metabolic aberrations in animals and supported the healing of injured kidneys.

In this study, we used a mixture of amino acids, herbal extracts and excretion stimulants (Carniforcyl Aqua, Virbac Vietnam) for assessment of its supportive effects on the health recovery of striped catfish via organ and tissue examinations. Additional microbiology tests on re-isolation of *E. ictaluri* from liver and kidney samples were carried out for further confirmation of full recovery of fish after feeding with this mixture.

Tank and fish preparation

Healthy striped catfish fingerlings (12.6±0.13g/fish) were used for this study. Fish were acclimated for a week and checked to ensure that there was no infection of parasites (on gills and internal organs) and bacteria (*E. ictaluri and Aeromonas hydrophila*) before starting the study.

BNP challenge protocol

A bacterial strain *E. ictaluri* CAF258 from the strain collection at Can Tho University, Vietnam was used for this challenge. The bacterial strain was enriched in TSB at 28°C for ~48 hours, then the cells were harvested by centrifugation and washed twice with 0.9% NaCl sterile solution. Bacterial density was adjusted to ~10^9 CFU/mL via optical density measurement at 610nm and plate counts on TSA (Miles et al., 1938).

Perfect Solution for Aquaculture

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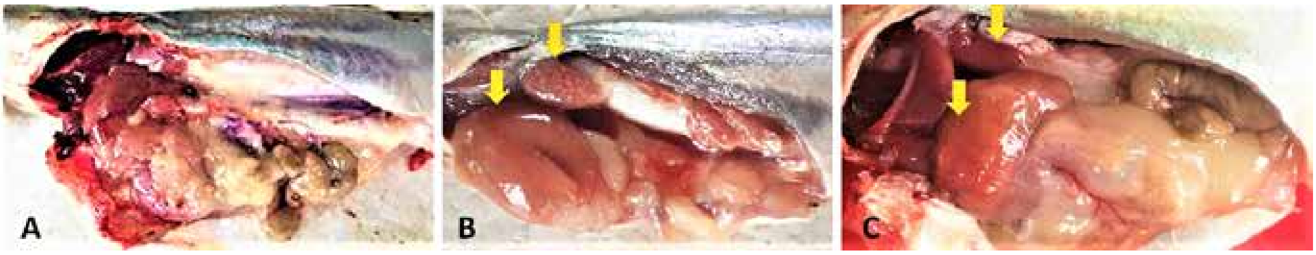

Figure 1. Clinical signs of internal organs of experimental fish with (A) normal internal appearance of unchallenged fish, (B) typical clinical signs of *Edwardsiella ictaluri* infection at day 3 of post-challenge with white patches in the kidney, and (C) internal appearance of challenged fish before feeding treatment, noted the pale liver in body cavity.

A preliminary test was carried out to determine the lethal dose of 50% (LD_{50}) and the challenge dose was calculated by immersion with bacterial suspensions. With a 500 fish/m^3 density in $2m^3$ tanks, 3,000 fish were challenged by 60-minute immersion. Fish were then distributed into 250L plastic tanks with 200 individuals in each tank (70% of freshwater with continuous aeration and temperature of 28–30°C). For the clinical follow-up, dead fish were recorded for signs of BNP-infection and removed from the tanks during the post-challenge period of 10 days. Bacteria from the fish liver (1 fish/tank) were isolated by culturing on a selective medium for *E. ictaluri* – EIA agar plates for infection confirmation. The remaining live fish were then ready for the experiment on different recovery applications/treatments.

Treatments

Six treatments were set up in triplicates with 80 individuals per 150L tank. Fish were transferred from challenge tanks and acclimated in treatment tanks for a day. Fish were fed with a commercial pelleted feed with/without supplementation of Carniforcyl Aqua (CA) via top-coating. Application doses were at 5 or 7.5mL/kg feed in various treatments. Top coating was applied with fish oil (20mL/kg feed). Application durations varied from 7 or 14 days.

Positive (PC, with challenge) and negative (NC, without challenge) controls were included by feeding the unsupplemented commercial pellets. During the treatment period, fish were fed *ad libitum* twice a day (12-hour interval). Water quality parameters were monitored daily with siphon and water exchange applied (~20% daily).

Before feeding with CA, 10 fish individuals were randomly collected for isolation of *E. ictaluri* on EIA and histology analysis. After feeding with the mixture for 7 and 14 days, 3 fish per tank were collected for the same analyses. Both liver and kidney of fish were fixed in 10% neutral buffered formalin fixative, then processed and stained with haematoxylin and eosin (H&E) for histology observed under a light microscopy using the method of Coolidge and Howard (1979).

Changes in clinical signs of experimental fish

On day 3 after the BNP challenge, sampled fish did not show obvious external clinical signs. Normal internal organs were observed in the NC (Figure 1A); however, internal clinical signs of typical *E. ictaluri* infection such as white patches on kidney, spleen and liver were noted in all collected samples subjected to the challenged test (Figure 1B). Surviving fish after 10 days of post-challenge showed few white patches in the internal organs (Figure 1C). All infected specimens displayed pale and swollen livers and empty intestines. Catfish is capable of excreting *E. ictaluri* after a disease infection (Nusbaum and Morrison, 2002); however, damages in internal organs were still observed as pale liver and kidney conditions.

On feeding with the mixture for 7 days, the liver of infected fish still looked pale in all challenged groups whilst the kidney showed a healthy/normal colour, and the intestines were full of feed. This indicates that fish ate normally after the treatment duration. Samples collected from groups fed with treatment diets for 7 days (both 5 and 7.5mL/kg feed)

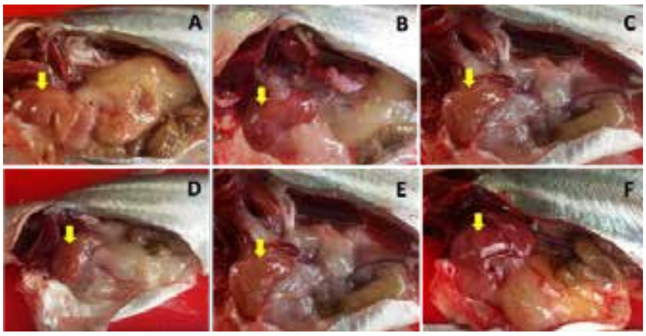

Figure 2. Challenged fish at the end of the experiment from treatments with Carniforcyl Aqua (CA) at (A) 5mL/kg feed for 7 days, (B) 5mL/kg feed for 14 days, (C) 7.5mL/kg feed for 7 days, (D) 7.5mL/kg feed for 14 days, compared with normal feed (E) positive control and (F) negative control.

still had organs with a pale colour (Figures 2A and C) in comparison to a pale colour in PC (Figure 2E) and a normal colour in NC (Figure 2F). After 14 days of CA additions, all collected fish samples looked healthy from the outside, and the fish liver looked as healthy as the NC fish (Figures 2B, D and F).

Without treatment, clinical signs of internal organs of fish with *E. ictaluri* infections were previously reported as chronic systemic damages on channel catfish although fish were tested negative for *E. ictaluri* after the outbreak (Shott et al., 1986). Infections on both channel catfish and striped catfish by *E. ictaluri* were similar on a histopathological point of view (Pirarat et al., 2016). In this study, the supplementation of the mixture at either 5 or 7.5mL/kg feed for 14 days drastically improved the condition of internal organs shown by both appearance and histology.

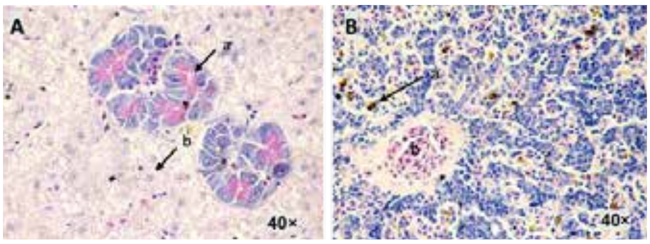

Figure 3. Histology of liver and head kidney of healthy fish from negative control (H&E stain).
A. healthy liver (40X) with exocrine pancreas (a) and liver cells (b).
B. healthy head kidney (40X) with melano-macrophage cells (a) and arteries (b).

Histopathology of experimental fish

Histological sections of fish samples taken at day 3 of post-challenge from the NC showed apparently healthy liver with exocrine pancreas and liver cells (Figure 3A), and healthy kidney with a lot of melano-macrophage cells and arteries full of blood cells (Figure 3B).

In contrast, fish collected from *E. ictaluri* challenged groups displayed the typical histopathology of bacillary necrosis (Figure 4). Liver tissues of infected fish showed the lack of blood cells in artery lumen and there were structural changes at the site of white patches (Figure 4A). At a higher magnification, structural changes in the liver, discrete and unconnected liver cells were shown with enlarged cell nuclei and concentrated lymphocytes (Figure 4B). In the head kidney tissues of challenged fish, there were areas of structural changes with artery lumen that lacked blood cells (Figure 4C), and severe necrosis that can be seen at a higher magnification (Figure 4D).

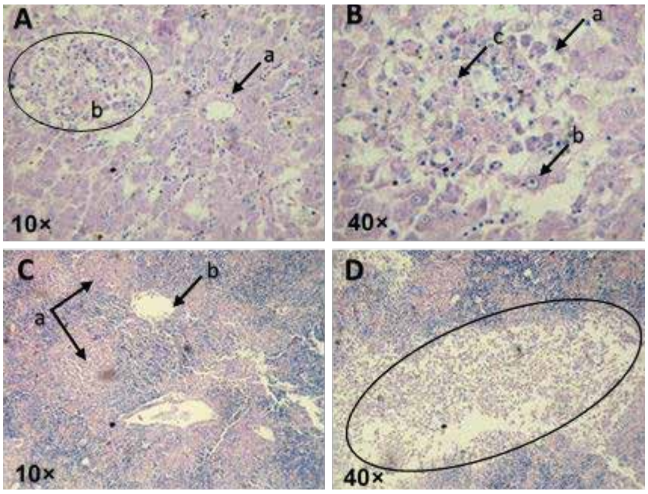

Figure 4. Histology of liver and head kidney of fish from *Edwardsiella ictaluri* challenged groups (H&E stain).
A. artery lumen in the liver (10X) lack of blood cells (a) and structural changes in the liver at the site of white patch (b).
B. higher magnification (40X) of structural changes in the liver showing discrete and unconnected liver cells (a), enlarged cell nucleus (b) and concentrated lymphocytes (c).
C. areas of structural changes in head kidney tissue (a) and artery lumen in the head kidney lack of blood cells (b) (10X).
D. severe necrosis in head kidney (40X).

Before recovery treatments, pale liver tissues of live challenged fish had concentrated lymphocytes, unconnected liver cells with irregular shapes, and degenerated cytoplasm (Figure 5A). However, the head kidney showed a healthy recovery naturally with melano-macrophage cells, red blood cells and lymphocytes (Figure 5B). After 7-day CA supplementations at 5 and 7.5mL/kg feed, the liver of challenged fish did not significantly recover (Figure 5A and 5B) and remained unchanged until the end of the experiment. However, 14-day application treatments (both 5 and 7.5mL/kg feed) with CA showed an enhanced recovery of liver of challenged fish with more connected and healthier liver cells, and more blood cells in the artery lumen (Figures 5C and 5D).

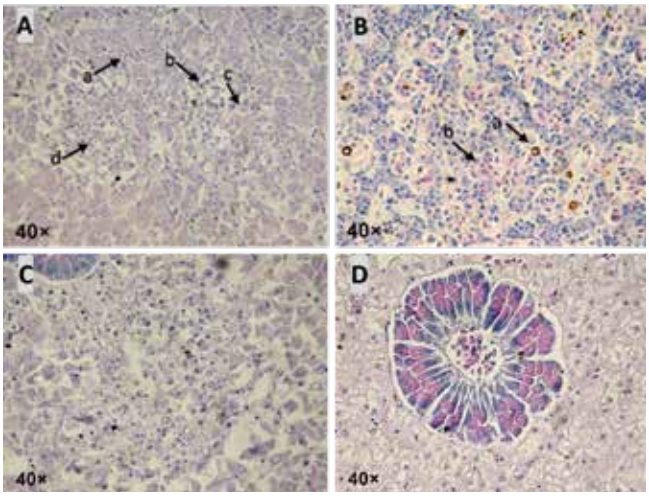

Figure 5. A. Histology of pale liver of challenged live fish without treatment diet showed concentrated lymphocytes (a), unconnected and irregularly shaped liver cells (b), eosinophilia in cytoplasm, nuclear degeneration (c) and vacuoles in the cytoplasm (d).
B. healthy kidney of challenged live fish without treatment diet showed melano-macrophage cells (a) and red blood cells and lymphocytes (b).
C. recovered liver tissue of survival challenged fish fed with Carniforcyl Aqua (CA) for 7 days, and (D) for 14 days with a full recovery.

Isolation of bacteria from liver and kidney

Results of *E. ictaluri* bacterial re-isolation on EIA are presented in Table 1. The bacteria can remain in internal organs of fish after a disease outbreak (Pirarat et al., 2016; Shott et al., 1986), which may introduce another outbreak in the same crop. It was reported that *E. ictaluri* possibly re-infected surviving fish due to low antibody titre after the first infection (Vinitnantharat and Plumb, 1993). High stocking density and environmental changes could also make the fish immune system vulnerable to a second outbreak. A previous study reported a case of *E. ictaluri* re-isolation after 30 days of post-infection (Pirarat et al., 2016), while another case documented the re-isolation of the pathogen in kidney and liver samples on day 26 of post-infection (Shott et al., 1986). A similar result has been found in this study as one of the samples of the challenged group (PC) without treatment showed the re-isolation of *E. ictaluri* after 26 days (from the challenge day). On day 7 with CA supplementations, a lower number of infected fish were found to still harbour *E. ictaluri* (11.1–16.6%, compared with 33.3% in the PC), and no fish sampled had the bacterial species on EIA at day 14. In super intensive striped catfish culture, accelerating the pathogen clearance may reduce re-infection issues and maintain good quality fish for harvest.

Experimental catfish with confirmed BNP infection showing quite normal appearance.

Treatment diets with doses of 5 or 7.5 mL/kg feed of Carniforcyl Aqua (CA)	Number of fish samples having colonies on EIA/Total number of fish samples			
	3 days after challenge	Before recovery treatment (Day 0)	7 days after feeding with CA	14 days after feeding with CA
CA 5.1	9/9	2/9	1/9	0/9
CA 5.2	9/9	2/9	1/9	0/9
CA 7.5.1	9/9	2/9	2/9	0/9
CA 7.5.2	9/9	2/9	1/9	0/9
Positive control (PC)	9/9	2/9	3/9	1/9
Negative control (NC)	0/9	0/9	0/9	0/9

Table 1. Bacterial isolation on EIA

Conclusion

The results of this trial noted that the liver and kidney of striped catfish had been affected heavily after the LD_{50} immersion challenge was applied. Applications of CA for 7 days supported the recovery of fish but had little effect in terms of liver and kidney status compared to the positive control. The results of supplementation at 5 and 7.5mL/kg feed for 14 days continuously did bring a fast improvement on appearance and condition of liver and kidney after the BNP infection. In addition, all treated diets enhanced the elimination process of the pathogen in fish since no *E. ictaluri* was recovered from the samples collected at day 14 of application. Further studies should be conducted to explore this beneficial effect of Carniforcyl Aqua, and its role in stimulating excretion on other aquatic species such as the white shrimp.


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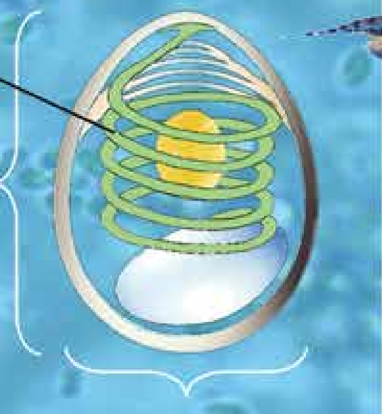
References are available on request

EHP: fact sheet

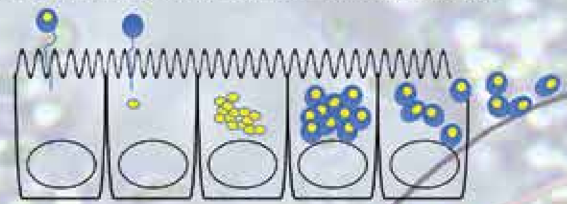
What is EHP?

EHP or *Enterocytozoon hepatopenaei* is a fungal microsporidian parasite that infects the hepatopancreas (hp) of tiger shrimp (*Penaeus monodon*) and whiteleg shrimp (*P. vannamei*) in Thailand and results in slow growth and, in chronic infections, mortalities. EHP is also known from Brunei, China, India, Indonesia, Malaysia, Philippines, and Vietnam. The report from Venezuela requires confirmation.



A spore of *E. hepatopenaei*

Inside the hp, a spore activates, releasing its polar filament, injecting the parasite's sporoplasm directly into a cell. Inside, the sporoplasm proliferates. Mature spores then develop which are released back into the gut damaging the cell which then sloughs away, the spores pass out in the faeces. Autoinfection is possible.



Shrimp become infected by ingesting spores from the water, from sediment, from eating EHP-infected live feeds (polychaetes, molluscs, frozen Artemia biomass etc) or by cannibalism.

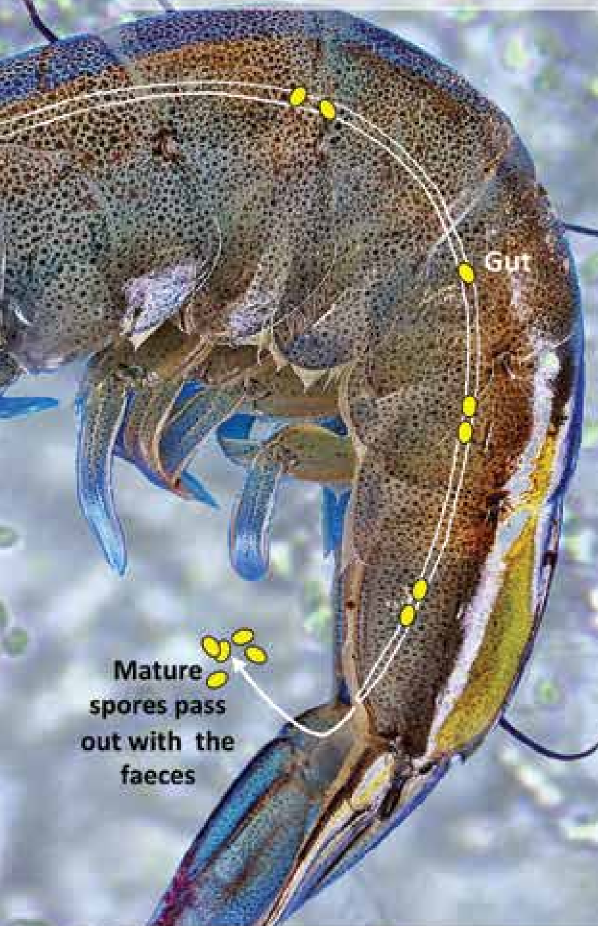
- How quickly infections develop will depend on the farm practices in place, the number of water exchanges made, the quality of the feed used etc;
- One of the biggest challenges is the circular movement of water between linked ponds which means a reservoir of infection is retained;
- SPF shrimp become infected within 2 weeks when cohabited with infected shrimp. Shrimp can become infected within one week when fed EHP-infected hp, and, within 15 days when exposed to pond soil. For earth ponds without a toilet where there is no removal of organic material and spores, infections can rise rapidly;
- PL that are PCR negative (-) but have a 20-30% infection in the hp might develop white faeces in 65-79 days. Note that the PCR reaction is to nuclear DNA coding for the spore wall; PCR detection of early stage infections are more challenging. For PL that are PCR positive (+) with a 50-60% in the hp, when transferred to ponds might develop white faeces within 30-44 days. PL that give a strong PCR positive (++) result and have a 40-90% infection in the hp might develop white faeces within 14-20 days.

How can I check for EHP?

- Infection can be checked by microscopic examination (at x100 oil immersion) of the hp and the gut of the shrimp;
- Infection can also be confirmed by molecular testing of the hp by PCR. Samples can be submitted live or fixed in ethanol to your local lab;
- Faecal samples from broodstock can also be tested by PCR;
- Regular health assessments are recommended. If there is a large difference in the size of PL, if PL feeding activity is less than expected, the number of lipids in the hp drops, growth and moulting slows, then these are also good indications of infection. The number of swollen tubules may provide an indication of how advanced infections are.

EHP infects the tubules of the hp causing cells to slough and impacts on the shrimp's ability to digest its diet. If shrimp are unable to digest their diet and repair the damage to lost tissue, the shrimp have a reduced appetite and slow growth.

EHP-infected shrimp may have a thin cuticle, white muscle as a stress response, black spots on their eyestalks, in their muscle tissue and along the hind gut.



How do I manage EHP infections?

In broodstock facilities

- Use EHP-free tested feeds only (e.g. SPF polychaetes) or use diets that have been preferably pasteurised or gamma irradiated, or have been frozen (i.e. 48 h @ -20°C to ensure that spores are destroyed);
- Disease checking of animals before their transfer into culture systems - this means only stocking EHP-free animals.

In hatcheries

- Soak tanks and associated pipework in 2.5% sodium hydroxide for 3 h, followed by complete dry out for 7 days. An increase of pH to >9 results in 90% of spores firing (i.e. once spores have fired, they are unable to infect host cells);
- Practice tight biosecurity;
- Disease check stock before their transfer into culture systems - this means only stocking EHP-free animals (i.e. destroy EHP positive batches);
- Conduct regular screening for EHP - swollen hp tubules may suggest an EHP infection;
- If PL eat less than expected, then check the hp and get a sample tested for EHP;
- Use of high-quality diets to promote shrimp health.

In grow-out ponds

- Observe proper stocking densities;
- Ensure effective disinfection of culture systems, pond liners, farm equipment and water;
- The ageing of water may reduce EHP infection;
- Only stock EHP-free PL. Destroy EHP-infected stock;
- Only buy PL from registered hatcheries;
- Keep pond bottoms clean - remove accumulating organic matter that could act as a spore reservoir;
- Move aerators to ensure effective water movement;
- If an infection is detected, give a high protein diet to help the shrimp's digestive capacity and recovery of the hp;
- Do not overfeed shrimp – energy spent in digestion will only weaken the shrimp;
- A wide range of natural products claim to control EHP infections including chitosan, various essential oils, herbal extracts, Spirulina, and, seaweed extracts – their performance needs confirming;
- Poly aluminium chloride is used to coagulate suspended organic material including spores causing suspended material to flocculate and to sediment which can then be removed / pumped away;
- Ensure any new water entering production ponds is treated to prevent re-infection.

Between grow-out cycles

- Drain ponds, check the sludge map and look for residual pools of water – move aerators for effective bottom cleaning;
- Using >15 ppm $KMnO_4$ or >40 ppm chlorine to inactive spores has been suggested (Aldama-Cano *et al.*, 2018);
- For earth ponds, adding calcium oxide (CaO) or quick lime at >6 tons hectare to quickly raise the pH from 8 to >11. Ponds must be completely dry, apply quick lime and then plough into the sediment to 10-12 cm depth, then moisten to activate the lime;
- Treatment of water before stocking with 18 g m^3 calcium hypochlorite to remove wild crustaceans.

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Moving forward with the green way of life!

Organic aquaculture is a young sector facing some wide, challenging issues.

By Carlos Rodríguez, Carlos López, Ewa Sujka and Lisa Collado

Source: The EUMOFA "EU Organic Aquaculture 2017.

In general, we could say that aquaculture reflects a seeking for sustainability interdependence of three "spheres" or classes of system: the economic, the social and the environmental, and we can consider organic aquaculture as being one step ahead when talking about sustainability and effective uses of resources, including social responsibility.

In the context of the 2030 Agenda, organic aquaculture can be moved to the next level to meet the United Nations' 17 Sustainable Development Goals (SDGs), with special attention allotted to SDG 14: Conserve and sustainably use the oceans, seas and marine resources for sustainable development.

Challenges can be myriad; problems appear to start from incubation and continue throughout the entire production cycle. Issues such as the difficulty in obtaining organic seedstock for certain fish and crustaceans species, veterinary treatment, welfare issues, or meeting all regulatory requirements for aquafeed and sources for sustainable fisheries, are subjects that need to be addressed in moving forward.

General rules on aquafeeds

Regarding organic feeds, given the early stage of organic aquaculture, there is an open debate and discussion on the balance that needs to be achieved between the fundamental rules in organic culture and the reality in the supply of the raw materials for aquafeeds.

Aquafeeds must be formulated according to the nutritional requirements of the farmed organisms (NRC 2011), and promote an animal's growth, health and welfare. On the other hand, organic products should ensure high quality of the final product, covering consumer expectations, and causing minimum environmental impact, including in terms of micro and macro nutrients.

Luckily, recent European Union (EU) regulations help to unify the criteria for independent certification bodies and international standards for products, substances and techniques authorised for use in organic fish production. Nevertheless, there are many issues that need further attention and solutions; the most important is a clear definition of concepts, which will help to avoid confusion, prevent potential market fraud and eventually work to increase trust of end users.

In the case of some species, carnivorous-omnivorous species such as penaeid shrimp and freshwater prawns, the EU production rule for organic aquaculture has set a maximum of 25% of fish meal and 10% of fish oil in aquafeeds.

In the case of the catfish (*Pangasius* spp.), a maximum of 10% fish meal or fish oil should preferably be derived from sustainable exploitation of fisheries as referred to in Article 5(o) of Council Regulation (EC) No 834/2007 and defined in Article 3(e) of Council Regulation (EC) No 2371/2002 of 20 December 2002 on the conservation and

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sustainable exploitation of fisheries resources under the Common Fisheries Policy (CFP) or organic feed derived from organic aquaculture sources.

Four principles in salmon production

However, in organic salmon production, under the umbrella of European laws and international standards, production must be based on specific principles, which can be extended to the production of other organic fish. Attention must be focused on monitoring a high level of animal welfare that respects the specific needs of the species:

1. The production of organic product from animals that have been raised on organic farms since birth or hatching and throughout their life.
2. Preservation of the health of the aquatic environment and the quality of the surrounding aquatic and terrestrial ecosystems.
3. Feeding aquatic organisms with feed from a sustainable exploitation of fisheries as defined by Article 3 Regulation 2371/2002. Conservation and sustainable exploitation of fisheries resources under the Common Fisheries Policy, or with organic feed made from agricultural ingredients from organic farming and natural non-agricultural substances as defined under (EC) 834/2007 Article 5.

A nutraceutical proposal

LIPTOSA S.A. (Spain) supports sustainability in different aspects, and when we detected an unsatisfied need in organic aquaculture, we start to develop solutions and tools for enhancing animal welfare in organic production, striving to cover possible gaps and enhance animal wellbeing.

Among the UN-United Nations' 17 Sustainable Development Goals (SDGs), special attention is on SDG 14 - Conserve and sustainably use the oceans, seas, and marine resources for sustainable development.

Recently, we have launched two products, under the scope of Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91.

Liptofry BIO, our first product which is a nutraceutical and phytobiotic to complement feed, is certified by European Union legislation for use in organic aquaculture, in all production phases. This nutraceutical proposal contains botanical compounds obtained from transformation of aromatic plants of organic agriculture. This product may be used continuously, improving zootechnical performance, and supporting the organism in neutralisation of oxidative stress, as it is a source of natural antioxidants.

Liptocitro MMM Bio is a complementary feed certified for its application in organic aquaculture during all production phases, helping to control the negative impact of parasitic infestations produced by trematodes and protozoa of cutaneous and branchial location and, in particular, the branchial parasitosis produced by monogeneans. Its nutraceutical properties also promote intestinal health, achieving an improvement in the nutrient absorption, offering a positive impact on zootechnical parameters, and being a source of natural antioxidants.

The basis for the standards

The concerns of organic aquaculture producers are on basic requirements to get the certification from the private certification body or national agency. However, independent of certification, phytobiotics developed by LIPTOSA's R&D department could be considered as an important device to ensure fish health and welfare under conditions of organic production.

Unwritten pages about sustainability and organic production, including organic aquaculture, are still awaiting a bright future; therefore, we should not lose focus of this niche market as an opportunity to diversify aquaculture production in some regions under organic sustainability concepts and achieving commitments with meeting the 17 Sustainable Development Goals.

Finally, it is interesting to note that under the current coronavirus situation, when all forecasts show a reduction in the volume of conventional salmon production, that of the organic salmon industry is stable and showing a better resilience to unfavourable conditions.

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Where there is krill, there is a way

A lifeline with this powerful feed supplement which improves nutritional composition and attractability of plant- based or reduced fish meal feeds, and therefore growth and health of white shrimp.

By Lena Burri and Geronimo Leonardi

The production of farmed shrimp is projected to grow by 5.4% between 2018 and 2021 - more than twice the recorded growth from 2012 to 2017 (Anderson et al., 2019). Despite this positive prediction, surveys identify that disease outbreaks and feed costs remain top concerns to further growth. Renewed outbreaks of Decapod Iridescent Virus 1 (DIV1) continue to threaten shrimp farming in China, according to seafoodsource.com and fish meal supply continues to decline whilst prices increase with demand (FAO, 2020; Sherrard, 2019). This directs new attention at plant-based aquafeeds as more sustainable alternatives (Tacon et al., 2010). To be a viable option, however, these alternative feeds need to measure up to traditional fish-based feeds in terms of zootechnical performance and environmental footprint of feed production and farming activity. Purely plant-based feeds suppress feeding stimulus because of antinutritional factors, a lack of chemoattractants and a poorer match between the amino acid profile of the feeds and the nutritional requirements of shrimp (Figure 1). Such feeds can severely impair shrimp growth and therefore curb production. Fortunately, the supplementation of plant-based shrimp feeds with krill meal has shown promising results by circumventing the nutritional deficiencies of a plant-based diet, whilst increasing shrimp growth and survival (Nunes et al.,2019; Suresh et al., 2011).

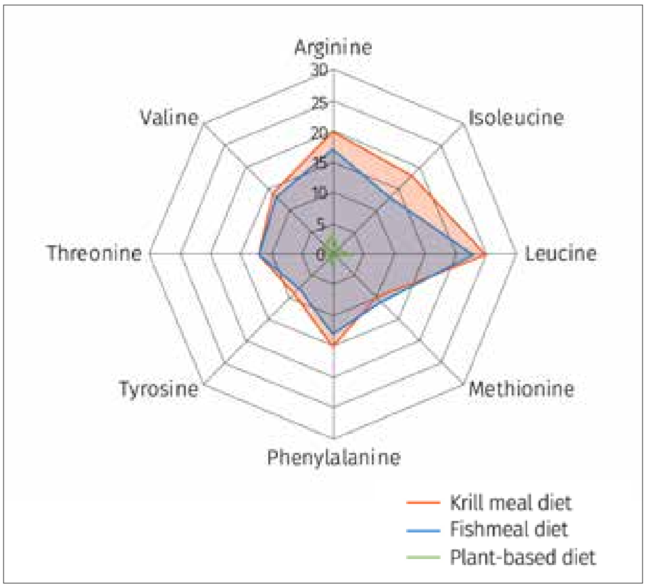


Figure 1. Essential amino acid profile (as g/100g-diet) of plant-based diets that do not contain fish meal or other animal-based protein sources (green line) with 3% fish meal (blue line), or with no fish meal and krill meal supplementation (orange line; krill meal inclusion at 110g/kg). Krill meal supplementation in no fish meal diets results in an equally high feed nutritional profile. Source: Moreno-Arias et al. (2018) for plant-based diet profile, and Nunes et al. (2011) for the fish meal and krill meal diet profiles.

Antarctic krill fisheries

Krill meal is produced from Antarctic krill (*Euphausia superba*) found in the Southern Ocean around the frozen continent. Krill survive their first winter living in the water column on the underside of sea ice where they feed on the available microscopic algae. Adult krill are shrimp-like in appearance, but, in contrast to their bottom-dwelling crustacean relatives, krill inhabit the open ocean. Here, krill aggregate in swarms of up to 20km in length where they feed on microscopic algae during the day and migrate to greater depths during the night to evade predators. Krill appear transparent with some red and green colouration. The red colour is caused by the carotenoid pigment astaxanthin embedded in chromatophores. The crustacean can change the size and intensity of the red spots to balance the need of UV protection closer to the ocean surface with higher transparency for camouflage (Auerswald et al., 2008). The green colour is observed in their digestive system and underscores their reliance on an algal diet. In fact, krill can consume up to 20% of their own body weight per day but can also survive for up to 200 days without food. During the Antarctic summers, when photosynthetic algae bloom, the sheer abundance of krill makes it one of the largest protein sources on Earth - a source eagerly sought by fish, whales, and humans alike.

Antarctic krill is exclusively caught in area 48 off the Antarctic peninsula and limited to 1% of the total estimated biomass in that area. Over the last 3-4 years, the total catch ranged between 230,000 to 390,000 tonnes annually; this represents only approximately 0.3% of the unexploited biomass. While annual variability in sea ice cover causes variation in recruitment, the estimated biomass of krill has increased from 60.3 million tonnes, as measured in 2000 to 62.6 million tonnes in 2018-2019, according to findings from the Commission for Conservation of Antarctic Marine Living Resources (CCAMLR). The conservative catch quota and trends in biomass ensure that krill stocks are amongst the best managed and underutilised marine resources to date.

Krill meal is the superfood among aquafeeds

Supplementation with krill meal has been known to improve aquafeed quality since well over a decade (Yoshitomi et al., 2007). Yet, the stagnating and even declining fish meal production from capture fisheries reinforces once again the need for a more sustainable protein source for the shrimp farming industry. Besides, a better understanding of nutrient requirements of shrimp has renewed the interest to look for alternative feeds and supplements that boost shrimp production and quality (Auerswald et al., 2008). Krill meal is uniquely suitable to meet these challenges.

After processing, the brownish-orange krill meal powder contains roughly 60% protein with a nutritionally well-balanced amino acid profile (Figure 1) and a long list of feeding effectors, including chitin, astaxanthin, nucleotides and phospholipids (Sabry-Neto et al., 2017; Derby et al., 2016). Such feeding effectors increase feed attractability and palatability, lower feeding latency time thereby reducing nutrient leaching and feed wastage, and consequently reduce overloading ponds with excess nutrients.

“On top of that, the main phospholipid contained in krill meal - phosphatidylcholine - not only supplies omega-3 fatty acids, but also choline. This is a rare but vital vitamin-like nutrient that supports neurotransmission and osmoregulation.”

Additionally, the dietary properties of several of these feeding effectors enrich krill meal to allow shrimp to cope with stressful situations (e.g. high plant-based diet, salinity or temperature changes). For instance, chitin may play a dual role as a feeding attractant and immunological stimulant to resist infections (Wang and Chen, 2008). Similarly, the antioxidant astaxanthin pigment has anti-inflammatory properties.

In contrast to fish meal, where omega-3 fatty acids are bound to triglycerides, these fatty acids in krill meal are neatly packaged with phospholipids. This in turn means omega-3 fatty acids from krill meal are effectively incorporated in tissue (Rossmeisl et al., 2012). On top of that, the main phospholipid contained in krill meal - phosphatidylcholine - not only supplies omega-3 fatty acids, but also choline. This is a rare but vital vitamin-like nutrient that supports neurotransmission and osmoregulation. This combination of chitin, astaxanthin, dietary phospholipids, omega-3 fatty acids, and other attractants allow shrimp to resist stressful farming conditions, while improving health and growth (Figure 2).

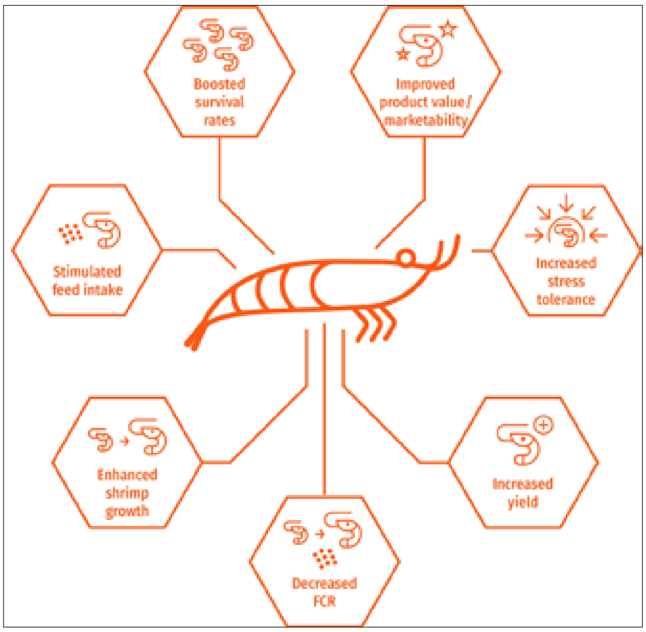


Figure 2. Benefits of incorporating krill meal into aquafeeds.

A case for krill

The importance of this potent combination of nutrients in krill meal as a dietary supplement for the shrimp farming industry has been made clear in several, recent scientific studies. Initially, the protein, lipid and nutritional values of fish meal, fish oil, soybean lecithin and cholesterol were proven to be replaceable by including krill meal with no costs to performance of white shrimp, *Litopenaeus vannamei* (Nunes et al., 2011). First, Suresh et al. (2011) showed that blue shrimp, *Litopenaeus stylirostris* fed a diet containing a high level of poultry by-product meal with a mere addition of 3% krill meal reached a comparable final body weight to those fed a purely fish meal-based diet. Later, Nunes et al. (2019) devised a study to compare growth performance of *L. vannamei* reared on a high plant-based diet with a stark reduction of fish meal content to only 3%. The diets were then supplemented with either 3% krill meal or six different marine chemoattractants. After 74 days, the final body weight was highest in shrimp fed the krill meal-supplemented diet (Figure 3). Feed conversion ratio was also lowest for shrimp fed with krill meal (1.31±0.05).

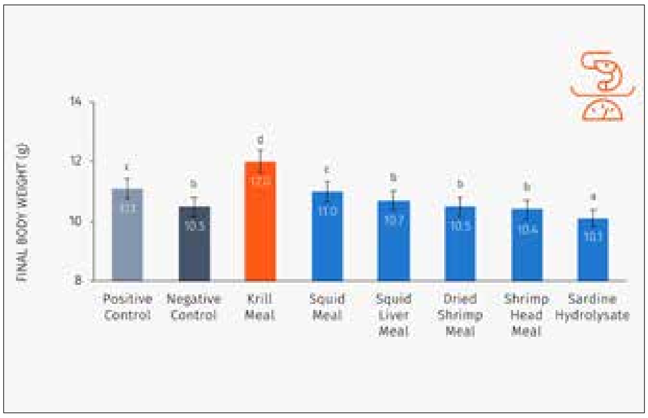


Figure 3. Final body weight of *Litopenaeus vannamei* juveniles fed with a soybean-based plant diet (negative control) supplemented with either 3% fish meal (positive control), krill meal or five other chemoattractants (except for sardine hydrolysate which was included at 5%) over 74 days. Different letters denote statistically significant differences between experimental diets ($P<0.05$). *Source*: Nunes et al. (2019).

In both studies the authors also assessed feed preferences. In the pairwise comparison, Nunes and colleagues fed shrimp with an excess of different feeds simultaneously twice a day over a total of 10 consecutive days. Leftover feeds were dried and compared. White shrimp clearly preferred krill meal and shrimp head meal supplemented diets over the other dietary supplements. Interestingly, in direct comparisons between shrimp head meal and krill meal, *L. vannamei* preferred the shrimp head meal-based diet. However, final body weight, growth, yield gained, and survival of the group fed with shrimp head meal were lower than that of shrimp fed a krill meal diet. A possible explanation could be the high presence of the biogenic amines cadaverine, putrescine, and tyramine in shrimp head meal, all known feeding attractants. Yet, these findings indicate that feed attractability alone does not necessarily make up for the poorer nutritional quality of the feed. Importantly however, these results emphasise the advantage of krill meal supplementation over other dietary supplements in a reduced-to-minimal fish meal-based diet.

Figure 4. Effect of krill meal inclusion (1, 2 or 3%) on *Litopenaeus vannamei* growth, gained yield and feed intake. Results are shown as percentage increase compared to the control plant-based diet without alternative ingredients or feed additives. Source: Sabry-Neto et al. (2017).

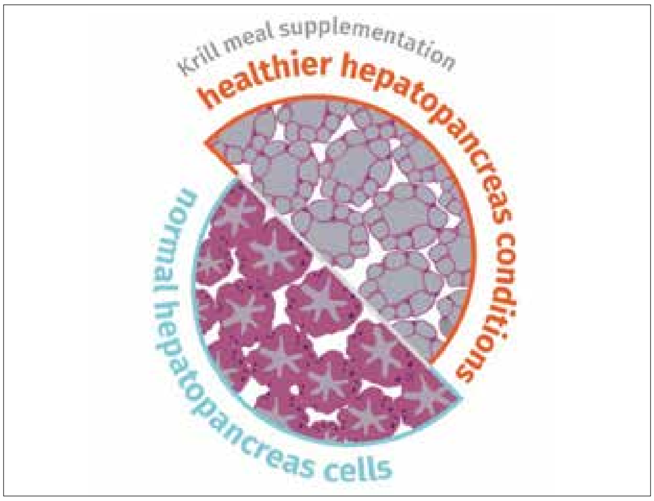


Figure 5. Differences between R- and B-cells in the hepatopancreas of shrimp fed non-krill meal supplemented diets and 2-6% krill meal-supplemented diets. The cell enlargement suggests improved digestive enzyme production, nutrient absorption, and lipid/glycogen storage functions.

A study by Sabry-Neto et al. (2017) went one step further– removing fish meal altogether. The researchers reared *L. vannamei* on either a plant-based diet with no krill added or four additional diets with gradually increasing levels of kill meal supplementation (0.5, 1, 2, and 3 %). Amongst these feeds the main dietary protein came from soybean meal- an all in all more sustainable source of protein than traditional fish meal diets. The addition of krill meal was predicted to overcome the anti-nutritional factors and nutritional deficiencies of such a pure plant-based diet. The results showed increased feed intake at 1% krill meal inclusion and significantly improved shrimp growth, with improvement in gained yield, feed intake and feed conversion ratio with 2% or higher krill meal inclusion. Shrimp growth and yield increased by an additional 16.3% and 20.1%, respectively, with a maximum survival of 99% at a 2% krill meal inclusion, when compared to the 1% krill meal inclusion (Figure 4). The study concluded that a minimum of 2% of krill meal in a plant-based diet enhanced all the major shrimp performance parameters with no costs to survival and health.

Burri et al. (2020) supports these findings further. The authors compared a high fish meal diet (20% tuna meal) with a highly reduced fish meal diet (7.5%; negative control) and, additionally, three levels of krill meal supplementation (2, 4 and 6%). Aside from the recurring trends of higher yields, increased weight gain and growth, when shrimp were fed krill meal, the histopathology of the hepatopancreas showed improved development of healthy star-like tubules with enlarged epithelial cells in shrimp fed with krill meal only (Figure 5). The different epithelial cells of hepatopancreas play important roles in nutrient absorption and storage as well as protein synthesis and, by extension, immunity. The superior performance of shrimp fed with krill meal enhanced diets may therefore stem from improved physiological development. Crucially, the authors highlighted that improved shrimp health and performance through krill meal supplementation can be achieved at no additional economic cost (Figure 6) and substantially reduced fish meal inclusion.

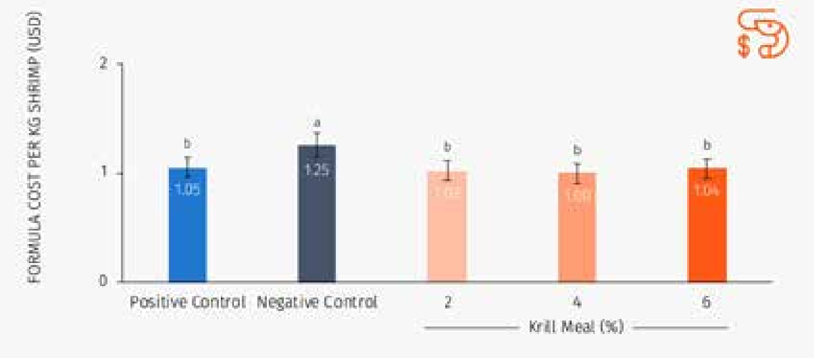
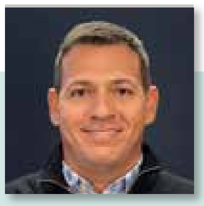


Figure 6. Impact of krill meal inclusion in feed cost in India, one of the world's key shrimp production areas (dietary treatments with 12% fish meal and different krill meal inclusion rates). Different letters denote statistically significant differences between experimental diets ($P < 0.05$). Source: Aker BioMarine Antarctic AS.

Conclusion

Krill is naturally abundant and nutritionally rich. Unsurprisingly, therefore, krill meal acts as a powerful feed supplement improving nutritional composition and attractability, and hence enhancing growth and health in shrimp. Krill meal supplementation throws a lifeline to an industry challenged by reduced fish meal availability at increased costs and nutritionally inferior plant-based feed alternatives. Moreover, increased yields at comparable feed costs result in a net profit in production and revenue (Figure 6). This was highlighted in a recent study by Leonardi and colleagues (submitted) that compared shrimp performance and economic viability of a control diet relying heavily on fish meal-derived protein and an experimental diet, that instead, used less fish meal and included high-protein krill meal. Both feeds were comparable in terms of feed price, but because the krill meal supplemented diet produced a much higher yield, the cost of the 3% krill meal supplemented feed (USD1.08/kg shrimp) ended up being lower than the cost of the feed with higher fish meal content (USD1.19/kg shrimp).

Since Antarctic krill is protected from overfishing by CCAMLR and Aker BioMarine Antarctic AS has obtained the Marine Stewardship Certification to prove sustainable fishing. QRILL™ Aqua is an attractive shrimp feed ingredient that maximises feed performance in a sustainable manner and also increases profitability of shrimp farming overall.



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Results are in from the first study on effect of krill meal on shrimp digestive gland

Scientists from Aker BioMarine, Kasetsart University (Thailand) and other research partners recently published an experiment on whether krill meal could successfully compensate for a reduction in fish meal in the white shrimp *Litopenaeus vannamei* shrimp diets. The study is also the first of its kind to specifically analyse the digestive gland condition, giving a unique perspective on nutrient digestion and absorption.

Below, **Dr Lena Burri,** Director R&D Animal Nutrition and Health at Aker BioMarine, shares an insider's perspective on the findings.

Dr Lena Burri is committed to shrimp nutrition and how to improve animal health with a strong focus on omega-3 fatty acids, phospholipids and astaxanthin. In her current position at the Norwegian krill company Aker BioMarine, she is involved in several research projects and has obtained extensive knowledge on nutrients from krill that she has published in various articles. Burri has a MSc from the University of Basel and a PhD from the Ludwig Institute for Cancer Research, both in Switzerland. Post-doctoral positions included stays at Melbourne University, Australia; University of British Columbia, Canada and University of Bergen, Norway.

Among the aquaculture research community, why is there so much focus today on finding alternatives to fish meal in shrimp diets?

Burri: Shrimp farmers are dependent on readily available and efficient shrimp feed, to ensure healthy and fast growing shrimp. The cost of fish meal has steadily risen in recent years, while supply has declined. This has created a greater demand for fish meal alternatives, but these need to be tried and tested to determine whether they are strong enough contenders to replace fish meal. What we see is that krill meal has stood out as a highly effective supplement in low fish meal diets, which has pushed us to deepen our understanding of its full potential.

How was this study conducted?

The study was carried out at the Kasetsart University in Bangkok under the supervision of Professor Orapint Jintasataporn. Here we divided shrimp into 15 tanks and assigned them to test diets. Feeding was three times daily for eight weeks. The diets tested included a high fish meal diet (20%), a low fish meal diet (7.5%) and three low fish meal diets with varying levels of krill meal added at 2, 4 and 6%.

What was the impact of krill meal when added to the low fish meal diets?

The groups consuming the low fish meal diets supplemented with krill meal all exhibited improved body weight, growth, yield and food consumption, at comparable levels to the group consuming the high fish meal diet. As little as 2% krill meal supplemented in the diet was shown to be successful in compensating for the reduction in fish meal.

Why did this study focus on the shrimp digestive gland, in particular?

The digestive gland or hepatopancreas, is a good indicator of shrimp health and growth performance. By looking into the differences between the R cells and B cells present in the various test groups, we were able to determine that these cells were positively enlarged among shrimp consuming krill meal in their diets.

What do enlarged R and B cells indicate?

Growth of shrimp is directly linked to the ability to absorb and digest nutrients in the body. R cells are known for their absorptive and lipid, glycogen and mineral storage function, while B cells are responsible for digesting nutrients and transporting them throughout the body. When these cells were enlarged in the hepatopancreas (among the krill meal test groups), it was an indication that krill meal had a positive impact on digestion and nutrient absorption in the shrimp.

You mentioned that one of the reasons that shrimp farmers are seeking supplements for fish meal is due to cost. From a cost perspective, is krill meal a viable alternative?

Yes, we see that from a cost perspective, supplementing just 2-6% krill meal into a low fish meal diet can be beneficial. On top of that, farmers have the added benefit of krill meal's nutritional advantages, including fatty acids, phospholipid and astaxanthin, all of which are associated with stronger shrimp growth performance. Farmers can raise healthier and larger-sized shrimp, with greater survival rates.

In just one sentence, can you summarise the overall conclusion of this study?

Krill meal has proven itself to be a highly effective supplement through this study, strong in nutritional value and in stimulating growth, which we now see may be due in part to its positive effects on the digestive gland.

More information: The study, "Effects of different feed inclusion levels of krill meal on growth and hepatopancreas morphology of Litopenaeus vannamei" was conducted by Lena Burri, Kjetil Berge, Srinoy Chumkam and Orapint Jintasataporn, on behalf of Aker BioMarine Antarctic AS and Kasetsart University, Thailand.

Advancing aquaculture with a yeast fermentate additive in aquafeed

Laboratory and field trials demonstrated dual-action effects of its bioactive compounds on the immune system and gut health of white shrimp and tilapia fry.

By Kok Leong Wee, Julie Gasper and Ning Widjaja

Since ancient times, fermentation has been recognised and used to enhance food preservation, quality, flavour and palatability as well as to impart anti-microbial properties to food. Fermentation is critical in the production of many of the foods that we enjoy today. For example, the bacteria *Lactobacillus* sp. is commonly used to produce lactic acids for cultured cheese products while yeasts, such as *Saccharomyces cerevisiae*, generate the alcohol in beverages or carbon dioxide in bread. However, fermentation offers many other additional benefits.

Fermentation is the process by which sugar is metabolised by organisms such as bacteria, moulds and fungi, to release energy. As part of this process, numerous metabolites are produced; these end products of fermentation have been touted as beneficial nutraceuticals. The metabolites, also described as "fermentate", include AMPs (anti-microbial peptides), bacteriocins, beta-glucans, biosurfactants, antioxidants, nucleotides, phospholipids, various vitamins including the B complexes, and volatile fatty acids.

Ongoing research suggests that these metabolites can have a positive effect on humans. Numerous studies conducted with EpiCor® fermentate, a postbiotic ingredient for human consumption made using the yeast *Saccharomyces cerevisiae*, showed that the fermentate helps support a healthy microbiome in the gut, and enhances the immune system. Because EpiCor is not highly processed or refined, it is a whole food yeast fermentate and contains a complex mix of nutrients and metabolites. Research has suggested that incorporating a yeast fermentate product into a daily regime can be a safe and effective way for people to live more healthy days.

In recent years, the use of fermentation products as ingredients or as additives in compound feeds has been extended to the animal feed sector. In animal feed applications, the benefits of using fermentation products include improving nutritive value by reducing the impact of anti-nutritional factors; enhancing immunomodulation; supporting gut health; and modifying gut microbiota to reduce the impact of pathogens.

Use of yeast fermentate in aquaculture

DVAQUA™ (Diamond V, USA) is a yeast fermentate product which supports the immune system and digestive health in farmed aquatic species. This natural* product is produced through a proprietary, multi-step anaerobic fermentation process using *S. cerevisiae*. It has been shown to help balance energy spent on maintenance and responses to stressors, while sparing energy to support growth and production. Research shows that DVAQUA has a dual-action effect; the bioactive compounds in this product support both the body's natural immune and digestive systems. With regards to immunity, it provides better disease protection with more efficient responses and faster recovery. It also provides better gut integrity while maintaining a healthy microbial balance. Ultimately, a feed that supports immune strength and digestive health helps to optimise the performance, growth and survival of cultured animals. (* natural as defined by AAFCO).

Enhancing immune responses

Improvements in growth and survival rates are supported by the enhanced immune responses in shrimp and fish. In a 2009 study on the white shrimp *Litopenaeus vannamei* conducted in Thailand, total haemocyte counts and phagocytosis rates were significantly higher when DVAQUA was included in the diet (Figures 1A and B). In addition, a dose-response effect was observed. Similar results were observed in various fish trials. However, while immune parameters are important indicators of a product's effectiveness, they may not be directly relevant in an aquaculture farm environment.

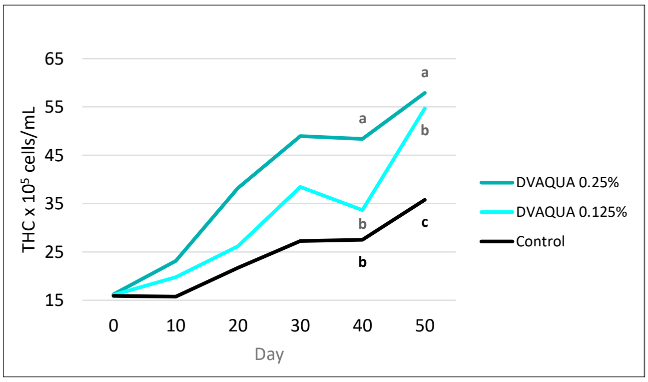


Figure 1A. Total haemocyte count of *Litopenaeus vannamei* fed diets supplemented with DVAQUA™ at inclusion rates of 0.125% and 0.25% compared with a control. abc denotes significant differences at $P<0.05$ (Tipsemongkol et al., 2009).

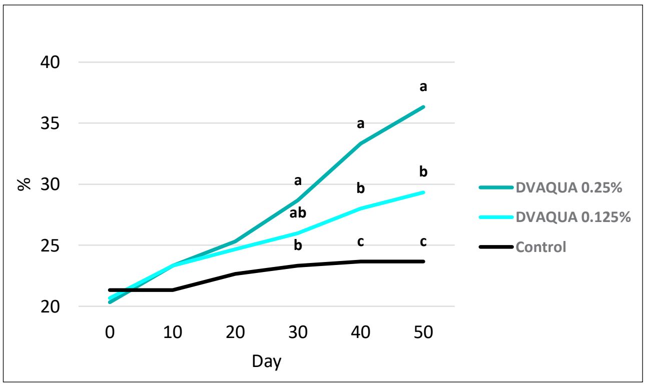
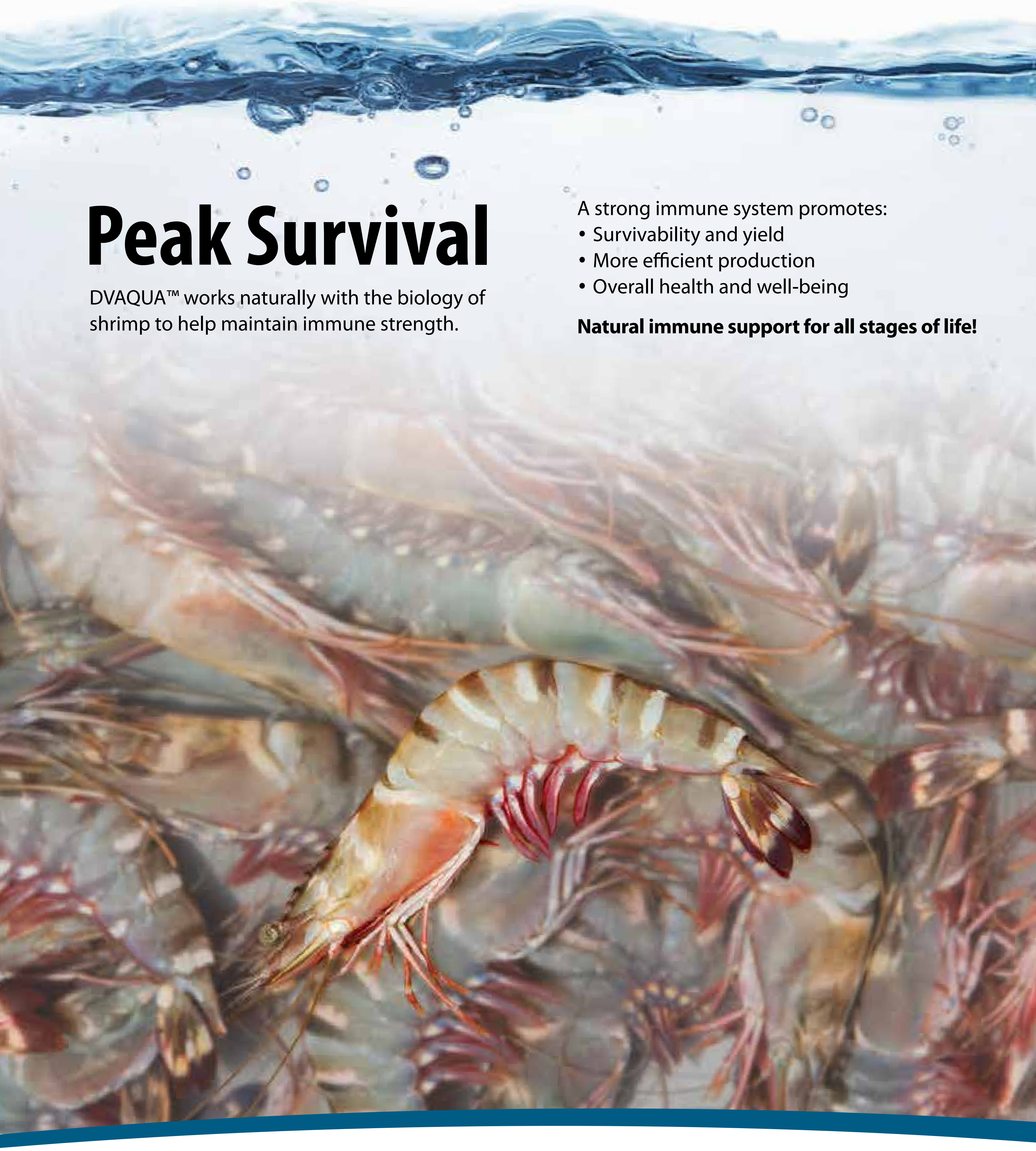


Figure 1B. Phagocytosis in *Litopenaeus vannamei* fed treatment diets supplemented with DVAQUA™ at inclusion rates of 0.125% and 0.25% compared with a control. abc denotes significant differences at $P < 0.05$ (Tipsemongkol et al., 2009).



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Disease challenges

Disease problems are among the most pressing challenges facing fish and shrimp farmers. The use of a yeast fermentate has been found to be most impactful in overcoming these disease challenges. It can help to improve intestinal function and balance the immune system, ultimately increasing the survival rates in fish and shrimp. Research shows a balanced immune system correlates with health and disease resistance as well as yield and survival. Healthy animals have better growth rates, and hence increased production and profitability.

For example, *L. vannamei* were challenged with white spot syndrome virus (WSSV) after 52 days of feeding either a control diet or a diet containing the yeast fermentate. The shrimp fed the latter diet had survival rates significantly higher than shrimp fed the control. These results were supported by another study which evaluated the impacts of *Vibrio harveyi*. Significant improvements in both immune response and survival were noted in shrimp fed diets supplemented with DVAQUA (Figure 2).

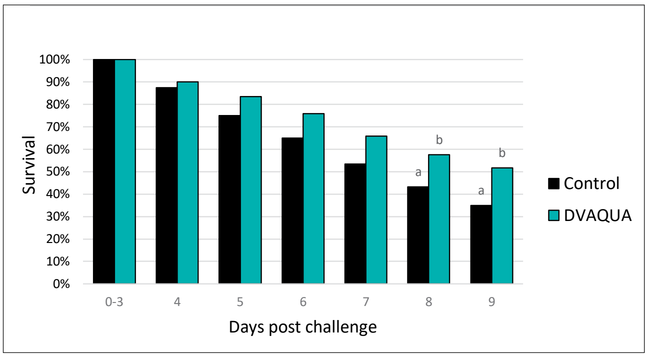


Figure 2. Survival of *Litopenaeus vannamei* after challenge with WSSV. ab denotes significant differences at $P<0.05$ (Kangsen and Zhen, 2006).

Results for a 2015 tilapia hatchery field trial showed that when fry (0.5g) were challenged with *Streptococcus agalactiae* with an intraperiotoneal injection at 2.49 x 10^8 CFU/fish, the group fed a diet supplemented with yeast fermentate showed greater resistance to infection compared to fish fed the control diet (Figure 3).

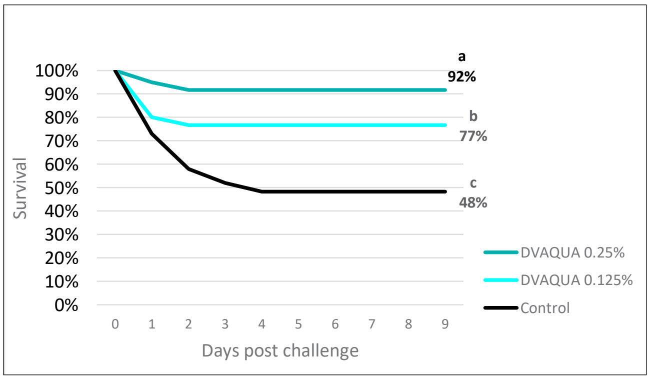


Figure 3. Cumulative survival rates (%) of tilapia *Oreochromis niloticus* fry after a *Streptococcus* challenge. Final survival rates are given for day 9. abc denotes significant differences at $P<0.01$.

Laboratory versus pond trials

Laboratory trials do not always represent actual conditions in a farm. On-farm validation of results from controlled laboratory research can provide farmers with more reassurance on the effectiveness of the product. In the case of DVAQUA, laboratory trial results have been validated at a commercial shrimp farm in Chantaburi province, Thailand. Over 120 days, shrimp were subjected to two treatments, with each treatment replicated four times. Shrimp in treatment 1 were fed a commercial feed (control), while those in treatment 2 were fed the same commercial feed with DVAQUA added. Not only did growth improve in shrimp fed diets supplemented with the yeast fermentate, but this was accomplished while also improving feed conversion ratio (FCR) and survival, resulting in a total production increase of more than 7% (Table 1).

	Control	DVAQUA	Difference
Production (kg/ha)	13,217^{a}	14,251^{a}	7.8%
Mean final weight (g)	18.0^{a}	17.4^{a}	-3.4%
FCR	1.75^{a}	1.64^{a}	-6.3%
Survival (%)	73.3%^{a}	82.1%^{a}	12.0%

Table 1. Results of a field trial in Chantaburi province, Thailand comparing *Litopenaeus vannamei* fed a commercial feed supplemented with 0.25% DVAQUA™ against a control. Mean values within the same row sharing the same superscript are not significantly different at $P<0.05$ (Tipsemongkol et al. 2009).

With the tilapia, similar improvements in performance were observed. In a commercial tilapia hatchery in Sao Paulo, Brazil, tilapia fry (average weight, 14mg) were fed either a commercial feed or a commercial feed with yeast fermentate added. The fish were stocked into eight hapas (700 fry per hapa) and harvested after 27 days of culture. Again, a significant difference in survival (+11%) and a beneficial reduction in feed conversion rates (-8%) were observed for tilapia fed the diet containing DVAQUA yeast fermentate (Table 2).

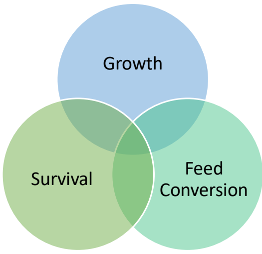
	Control	DVAQUA	Difference
Final weight (g)	0.25	0.25	0.0%
FCR	1.46^{b}	1.34^{a}	-8.2%
Survival %	80.1^{b}	89^{a}	11.1%

Table 2. Results of a 2015 field trial in Sao Paulo, Brazil comparing *Oreochromis niloticus* fry fed commercial feed supplemented with 0.125% DVAQUA™ against a control. ^{ab}denotes significant differences at $P<0.01$.

From these trials we can see that supplementation with yeast fermentate from *S. cerevisiae* ameliorated the growth, feed conversion, and survival rates of shrimp and fish. Aquatic animals accepted the additive with productive and positive outcomes.

Interaction of growth, FCR, survival rate and production

By supporting an efficient and balanced immune function, more energy can be spared for growth. Additionally, supporting digestive health allows for efficient utilisation of feed for growth, too. While strong growth results are what every farmer wants, these cannot be looked at as standalone markers. Larger shrimp will not always result in greater productivity – or profitability – if there are significantly fewer shrimp surviving or if more feed is used due to inefficient feed conversion. Each of these factors should be considered to understand the full impact of a feed additive.



Multiple research studies have shown that shrimp and fish fed diets containing the yeast fermentate consistently demonstrate better growth performance and feed conversion efficiencies. For example, recent shrimp studies from 2018 and 2019 conducted with a yeast fermentate (DVAQUA) resulted in average net weight improvements over 6% versus the control while feed conversion showed a beneficial decrease of more than 4%.

Product consistency

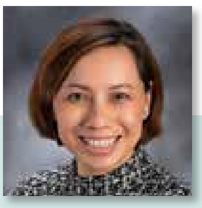
In order to ensure consistent efficacy in farms, all feed additives being considered for inclusion in compound feed should be reviewed for uniform quality. The consistency of fermentates can be impacted by raw material and ingredient quality, plant operations and procedures, and manufacturing experience.

Diamond V has spent over 70 years in developing and refining its proprietary fermentation process used to make DVAQUA. This long history has resulted in strict selection criteria for raw materials and well-defined operational processes. All these have led to the manufacture of this yeast fermentate which is both safe and consistent from batch to batch. The company's United States manufacturing facility has achieved the standards required for various third-party quality and safety certifications, including GMP+ and HACCP.

Feeding the future

Today, the beneficial effects of yeast fermentate in feed applications have become clear through numerous studies like those described above. By pairing this ancient practice with modern manufacturing technology, performance, growth, and survivability can all be optimised to help propel the aquaculture industry forward into the future.





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A new generation of feed palatability enhancers designed for carnivorous fish species

This is a game changer to standardise feed palatability in nutritionally balanced feeds, independent of the quality of other dietary raw materials, and to improve growth performance under standard and stressful conditions.

By Paul Seguin, Vincent Fournier, Mikael Herault and Fabio Soller

Feed palatability is a critical aspect of feed since it can enhance the growth performance in fish and shrimp; it is also the first criterion checked by farmers when feeding their animals. As feed palatability will affect the level of feed intake, it will impact all the nutritional, health and environmental key performance indicators of farms as well. For decades, feed palatability has been taken care of by formulating feeds with high levels of fish meal. With the continuous decrease of dietary fish meal in feed formulation for economical and sustainability reasons, we need to retain optimal feeding behaviour with an alternative source of attractants, especially when using plant based raw materials as alternatives to fish meal in carnivorous fish feed formulations. Other marine meals such as krill and squid meal have been used to balance the attractiveness of these new low fish meal aquafeeds but availability, consistency, price and sustainability of such solutions are limiting factors. Moreover, palatability of such raw materials still has room for improvement.

Enhancing feed palatability results in high performance in fish and shrimp during the rearing cycle. Undeniably, farmed animals face many stressful periods, from the beginning of their life to harvesting, involving activities such as weighing, grading, transferring, vaccination, veterinary treatments, feed change, and water quality management. All these interventions affect feed consumption. It is therefore possible, and highly recommended, to improve farm performance by offsetting these periods of low feed intake and stimulating animal feeding behaviour.

Carnivorous fish are among the most demanding species in terms of feed palatability. Kasumyan and Doving (2003) showed that these species are very sensitive to very low molecular nitrogen compounds (LMW: free amino acids, amino acids derivatives, peptides, amines etc.) usually found in the soluble fraction of marine protein raw materials but not in alternative raw materials (Figure 1). Thus, decreasing marine protein meals in a feed formulation will affect the concentration of the LMW compounds, hence affecting feed performance.

A new generation of palatability enhancers

Diana Aqua specialises in the development, testing and marketing of palatability enhancers for the aquaculture industry. The company has launched a new range of very innovative products (Extrapal range) made from proprietary protein hydrolysates. These palatability enhancers are characterised by a very high level of hydrolysis resulting in a high content of free amino acids and specific peptides, combined to selected ingredients, proven to boost the palatability performance of feeds designed for carnivorous species. These unique palatable solutions have been developed from different raw materials, mostly marine co-products.

Beside the formulation of a palatability enhancer, application is also a key factor to reach the best cost/performance ratio. Indeed, fish are characterised by a quick feed intake, with low or no chewing before ingesting the feed. So, palatable compounds must be released in water as soon as pellets touch the water to trigger fish taste receptors as fast as possible. In the same way, they must be detected by the taste buds once the pellets reach the fish's mouth. Consequently, coating the pellets remains the most recommended application

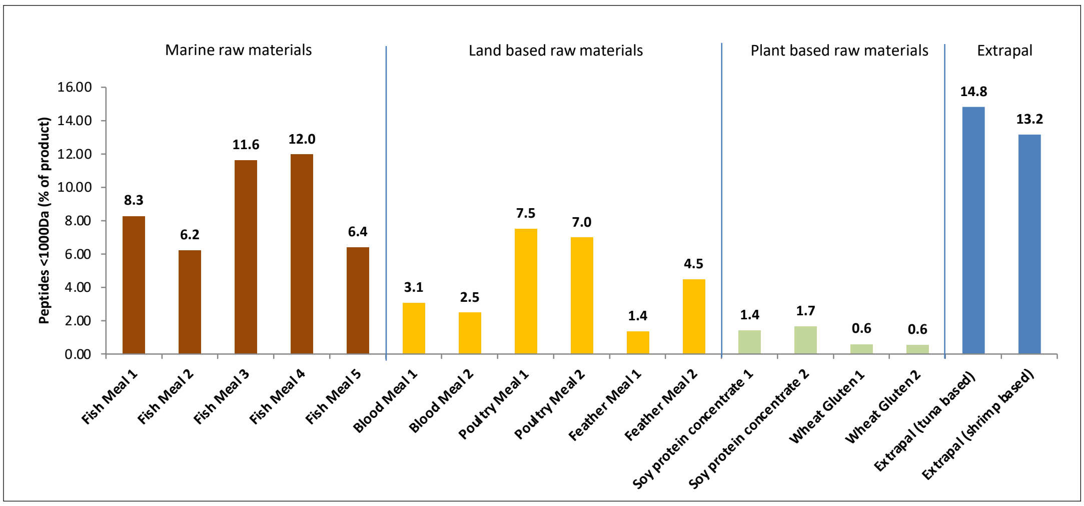

Figure 1. Levels of peptides less than 1,000Da in different raw materials utilised in aquafeed formulations and in two Extrapal liquid palatability enhancers (% of product).

to attain these objectives. Liquid forms also represent an interesting alternative to powder forms because active compounds are already solubilised and will be distributed homogeneously on the surface of pellets during the coating step after feed extrusion. In addition, top coating modulates product application depending on pellet size.

Diana Aqua's expertise in feed palatability has grown as a result of years of research and trials conducted with several different fish (and shrimp) species. Below, we present the most relevant results recorded in some stressful testing conditions, which fish would encounter in the farm environment.

How palatability enhancers can meet feed formulation and farm challenges.

Performances improvement of low palatable feed formulations

A farmed fish will encounter on a daily basis, issues linked to feed formulations. During the growth cycle, fish will be fed feeds of different formulations that may come from different feed suppliers and/or various qualities of raw materials. This is especially true with regards to quality and quantity of fish meal which tend to decrease as the fish grows. Fish will require time to adapt when switching to new feed formulation, initially resulting in lower feed intake, decreased growth and feed performance. Figure 2 illustrates the performance of a palatability enhancer in a carnivorous fish (European seabass) fed a 10% fish meal diet after a first conditioning step with a 50% fish meal diet. Results showed that application of a palatability enhancer significantly improved the performance of the 10% fish meal diet with a good dose response, to reach almost 90% of the growth performance when fed a high fish meal diet. This was achieved despite a decrease of 80% of dietary fish meal.

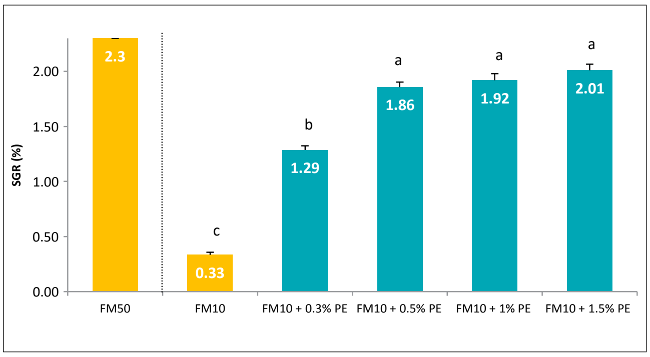

Figure 2. Transition from a high fish meal (FM50) to a low fish meal formulation (FM10) in European seabass *Dicentrarchus labrax* (PE: palatability enhancer; SGR: specific growth rate; values are means of three replicates ± standard error of the mean (SEM); trial duration: 18 days).

Performance of Extrapal solutions was also assessed in two warm water carnivorous species, in the context of fish meal reduction. In Asian seabass the addition of a palatability enhancer helped to reduce dietary fish meal inclusion from 15% to 8% with no significant loss in fish performance (Figure 3). Similarly, growth performance was not affected when red seabream was fed a 25% fish meal diet coated with 2% of a liquid palatability enhancer, in lieu of a 50% fish meal diet.

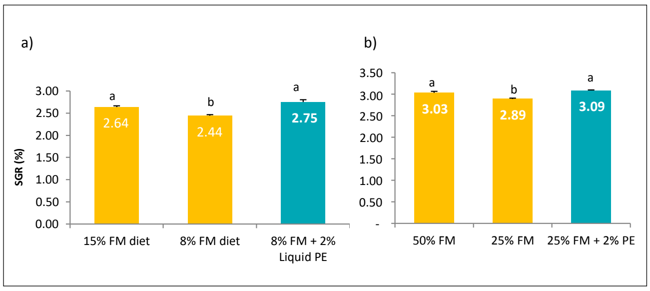

Figure 3. Specific growth rate (SGR, %) of Asian seabass *Lates calcarifer* (a) and red seabream *Pagrus major* (b) fed a low fish meal diet (PE: palatability enhancer; duration of trials: 12 weeks; values are means of three replicates ± SEM).

Another interesting application of a palatability enhancer is when medicated feeds are used. Application of Extrapal products helped to mask the unpleasant taste of bitter molecules to enhance feed intake and so improve the efficiency of veterinary treatments (Figure 4).

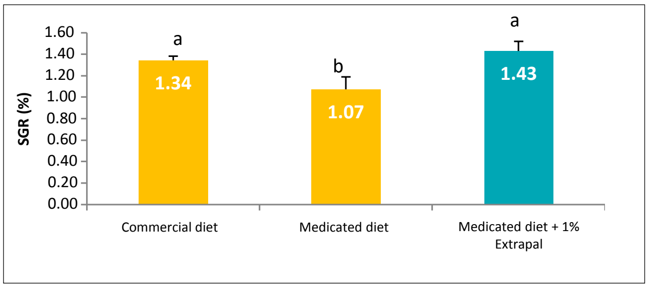

Figure 4. Specific growth rate (SGR, %) of Atlantic salmon *Salmo salar* fed a medicated diet, coated or uncoated with a palatability enhancer (values are means of three replicates ± SEM).

Stimulation of fish feeding behaviour during stressful events

In order to show the proof of concept of performance of a palatability enhancer during stressful rearing conditions, a meta-analysis was carried out on results from 12 different trials conducted with the European seabass grown in two similar facilities: one located in a quiet environment and the other in a noisier and busier location. For each trial, the same batch of fish and feeding conditions were used. The results of this meta-analysis showed that fish grown in a stressful environment had lower growth performance compared to the quieter one. However, the application of a palatability enhancer showed a recovery in growth performance, i.e. at the same level of the fish reared in the quiet facility (Figure 5). These results confirmed that feed palatability is an important and interesting tool to improve fish performance during and after stressful periods.

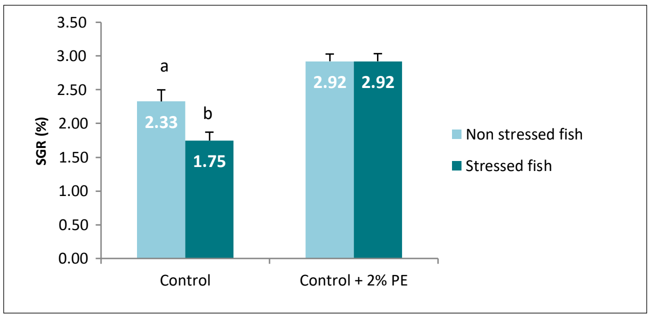

Figure 5. Specific growth rate (SGR, %) of European seabass *Dicentrarchus labrax* fed diets with and without a palatability enhancer, and reared either in a quiet or stressful environment (PE: palatability enhancer; values are means ± SEM of 12 trials of 15 days).

Stimulation of fish feeding behaviour during sub-optimal environmental rearing conditions

Since fish are ectotherm organisms, their feeding behaviour and metabolism are highly dependent on outdoor temperatures, with low temperatures resulting in lower zootechnical performance. This is particularly well represented in Figure 6 where growth performance of European seabass drastically fell when fish moved from 20°C to 13°C water temperature conditions. Again, the application of a palatability enhancer allowed fish feeding behaviour to be stimulated, hence resulting in an increase of growth of 15% at the end of the trial. This dietary application is essential to design dedicated functional diets that would help farmers to cope with cold seasons/ temperatures.

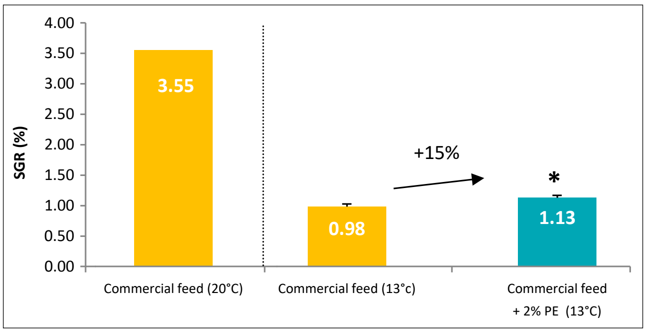

Figure 6. Specific growth rate (SGR, %) of European seabass *Dicentrarchus labrax* reared in cold water conditions and fed diets with and without a palatability enhancer (PE: palatability enhancer; duration of trial: 44 days; values are means of three replicates ± SEM).

Raising the bar for palatability with benchmarking relative to prime fish meal and standard fish hydrolysate

Diana Aqua has been developing and testing products for their palatability benefits for over a decade in its testing centres in Europe, Latin America and Asia. The development of a unique and sensitive method to assess palatability enables the company to benchmark and to rank the performance of its products to raw materials and ingredients commonly found in the aquafeed market. Figure 7 shows the palatability level of one palatability enhancer manufactured and formulated from shrimp co-products compared to high quality fish meal and a standard fish hydrolysate in a powder form. It clearly shows that the Extrapal liquid palatability enhancer outperforms the best fish meal quality and powder hydrolysate commonly included in feed formulations to stimulate feed intake in many aquaculture species.

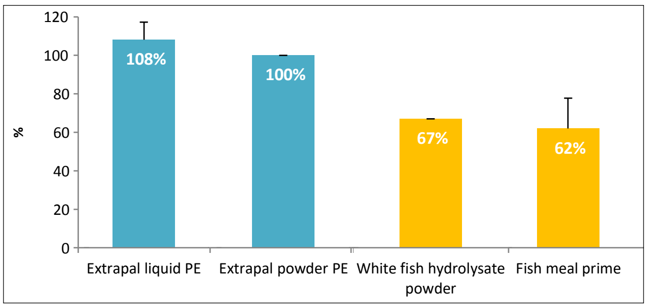

Figure 7. Palatability performance of a liquid palatability enhancer compared to a high quality fish meal and to a white fish hydrolysate in a powder form (results normalised to the performance of a reference powder palatability enhancer). Species: European seabass *Dicentrarchus labrax*. PE: palatability enhancer. Values are means of three replicates ± SEM.

Benefits of palatability enhancers to feed manufacturers and farmers

For feed manufacturers, the utilisation of a feed palatability enhancer provides flexibility in terms of feed formulation. Its use is essential to enhance and standardise feed palatability, independently of the quality of other dietary raw materials or ingredients, and their variability from batch to batch. Thus, for feed formulators making dietary changes is easier, from a wider choice of raw materials or fish meal grades. Obviously, it is worth noting that such products are not designed to offset serious dietary nutritional deficiencies, which remain the responsibility of feed formulators.

On the other hand, farmers can observe much more consistent feeding behaviour day by day, cage by cage and cycle by cycle, resulting in a very smooth evolution of feed intake along the rearing cycle with no reduction of fish growth. The recovery of high feed intake following stressful events has been quick. There was also less impact with the transition between feeds (feed size, formulation or supplier). This is particularly visible when fingerlings are transferred from hatcheries to ponds or cages. By reducing the intensity and duration of periods of lower feed intake, it results in shorter rearing cycle with more homogeneous fish sizes within, and between, cages or ponds. Side benefits are lower dominance or cannibalism risks, also making healthier fish to cope with further unavoidable stressors.

Conclusion

The introduction of this new generation of palatability enhancers is a game changer for the aquaculture industry. Aquaculture players can now rely on a functional dietary solution to help them improve and standardise fish and feed performance. The combination of two innovations related to feed formulation and top coating application provides feed manufacturers with ways to differentiate their products in a very competitive environment, guaranteeing their customers high and consistent feed palatability and performance that farmers are able to assess from first feeding to harvest.

Feed and farm sustainability will also be positively impacted by such utilisation of palatability enhancers. Extrapal range of products open more opportunities for feed formulators to experiment with new raw materials while reducing feed waste and fish loss during critical periods, resulting in an improved environmental footprint in fish farms.

Reference

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Diana Aqua-Aquativ is the aquaculture division of Diana, part of Symrise AG group.

Mycotoxins in aquaculture

Often underestimated and overlooked by industry, the mycotoxin threat increases with the move towards a sustainable approach with more plant-based ingredients in aquafeed.

By Sudhakar V. S. Govindam and Henry Wong

Mycotoxins are toxic chemical compounds produced by fungi which grow on agriculture crops and animal feedstuffs. Mycotoxins are diverse in terms of chemical structure and have toxic effects on animals. When consumed, mycotoxin contaminated feeds cause severe health or metabolic problems in farm animals and humans.

To date more than 500 different mycotoxins have been identified and most animal feedstuffs are likely to be contaminated with multiple mycotoxins. These toxins are primarily produced by the species that belong to the genera *Aspergillus, Penicillium, Fusarium* and *Claviceps*. Fungal growth and mycotoxin contamination occur in agriculture fields, during post-harvest handling and in adverse storage conditions. *Fusarium* spp., *Claviceps* spp. and *Neotyphodium* spp. generally grow on live crops in the field. *Fusarium* spp. produce some of the most common mycotoxins such as fumonisin, fusaric acid, trichothecenes such as deoxynivalenol (DON) or T-2/HT-2 toxins and zearalenone. Species of *Claviceps* and *Neotyphodium* produce ergot alkaloids in grains and grasses, respectively. *Aspergillus* spp. and *Penicillium* spp. are more common during storage, although they can grow on field crops, and produce mycotoxins such as aflatoxins, ochratoxins, citrinin, penicillic acid, patulin and mycophenolic acid.

There has also been reported incidence of *Aspergillus* mould contamination of fish meal (Mayahi, 2007). High moisture and high temperature conditions, improper storage, insect damage, drought and abnormal rainfall are some of the causes leading to fungal growth and subsequent mycotoxin contamination. Alltech conducted a worldwide mycotoxin survey during 2012-2015 by collecting 9,564 samples of feed ingredients (corn, wheat, barley, soybean) and formulated feed. The survey reported that 98.6% of the samples were contaminated with 56,191 discrete instances of mycotoxin contaminations and an average of six different mycotoxins per sample (Yiannikouris, 2016).

Mycotoxins are highly stable chemical compounds and can sustain high temperatures and pressure during feed manufacturing (Kabak, 2009). Bioaccumulation of mycotoxins in farm animals could pose a direct risk to humans (Danicke et al., 2013). The EU, with its commitment to the highest standards of food safety, has put some regulatory limits for aflatoxin and guidance values for DON, zearalenone, ochratoxin A, T-2 and HT-2 and fuminosins in products intended for animal feeding (Directive 2002/32/EC and European Commission, 2006).

Mycotoxin concerns in aquaculture

With the move towards a sustainable approach in aquaculture nutrition using more plant-based ingredients, feed producers and farmers should be aware of the potential threat of mycotoxin contamination. However, the mycotoxin threat in aquaculture and aquafeed has often been underestimated and overlooked by the industry.

Fish

The first recognised incidences of mycotoxicosis of fish occurred more than 55 years ago in trout hatcheries in the US when rainbow trout (*Salmo gairdneri*), fed pelleted diets that were prepared with cottonseed meal contaminated with aflatoxin, developed liver tumours (Halver, 1969). Jantrarotai and Lovell (1990) evaluated the toxicity of cyclopiazonic acid (CPA), a mycotoxin produced by *Penicillium*, on channel catfish (*Ictalurus punctatus*) and found it to be affecting growth. In another experiment by Manning (1998) on channel catfish, it was observed that 50mg of fuminosin B1 per kg of diet, significantly reduced the growth of the fish.

Recently a trial was conducted by Chinese researchers to evaluate the effects of dietary DON and aflatoxins on the growth performance, immune response and intestinal health in the turbot (*Scophthalmus maximus*). Both mycotoxins have profoundly reduced the nutrient utilisation rate and growth performance, suppressed immune response, affected the intestinal morphological structures, decreased the intestinal bacterial community diversity, decreased the abundance of potential probiotics and increased the quantity of potential pathogen (Yang et al., in press).

Organ damage and hepatic lipid accumulation in carp (*Cyprinus carpio*) is also reported after exposure to the feed borne mycotoxin, DON (Constanze, 2014). Finally, aflatoxin B_1 (AFB_1) reduces growth performance, physiological response, and disease resistance in tra catfish, *Pangasius hypophthalmus* (Rui et al., 2018). This collection of research data indicates the mycotoxin related pathological issues in aquaculture species.

Marine shrimp

In white shrimp *Litopenaeus vannamei*, growth and muscle were affected when fed diets containing 0.6ug/g of fumonisin B1 for 30 days (Miriam-Hiesu et al., 2015). When *L. vannamei* and *Penaeus monodon* were given a diet containing T-2 at 1.0–2.0mg/kg for up to 10 weeks, it was found that their digestive tract mucosa was severely inflamed (Supamattaya et al., 2006). A study conducted by Zhanrui et al. (2019) demonstrated that T-2 toxin, produced by a species of *Fusarium*, reduced growth and digestive activity, while promoting degeneration and necrosis of intestinal mucosal tissue.

Qiu et al. (2016), found that T-2 damaged the microstructure of shrimp hepatopancreas in a concentration-dependent manner and had a significant effect on alkaline phosphatase (AKP), glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activities. Deng et al. (2017) proved dietary exposure to T-2 significantly decreased ($P<0.05$) shrimp growth and survival rate compared with the controls. This research also showed a dose-dependent increase in reactive oxygen species (ROS), superoxide dismutase (SOD) enzyme activity and T-AOC at low T-2 exposures, along with associated histopathological changes in the hepatopancreas.


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Although there is research on the impacts of mycotoxins in aquaculture, farmers are still facing huge economic losses due to this silent enemy, since the symptoms of mycotoxicosis in aquaculture species are often unknown at the farm level. Producers may not be aware of the threat of mycotoxins, and even if they are, chronic intake of lower levels may go unnoticed. A possible example of such a scenario is white faeces syndrome and acute hepatopancreatic necrosis disease in *L. vannamei*, two of the most common disease problems in shrimp farming in Asian countries. Recent research trials in *L. vannamei* have proven the adverse effects of mycotoxins on digestive system, hepatopancreatic tissue and growth. When commercial toxin binders were used, the performance in the shrimp was enhanced (Qiu et al., 2016; Zhanrui et al., 2019; Mireya, 2017). So, feed producers in Asia must consider mycotoxin contamination of aquafeeds as a serious threat and proper mitigation strategies need to be considered to safeguard the farming community.

Mycotoxin management

At the feed mill, mycotoxin management begins from the screening of raw materials for mycotoxins. While screening, care should be taken on proper sample collection and appropriate advanced analytical techniques should be utilised for detection of mycotoxin type and concentration.

In recent times, there are many different methods for mycotoxin analysis. One analytical method that is user-friendly and has decreased the time required for accurate determination of mycotoxin content is the direct competitive enzyme-linked immunosorbent assay or ELISA. There are several other analytical techniques, such as immune-affinity column chromatography and high-performance liquid chromatography (HPLC), available for isolation and quantification of mycotoxins. The most sophisticated technique for the wide screening and accurate quantification of mycotoxin is ultra-performance liquid chromatographic (UPLC) techniques in combination with mass spectrometry (LC-MS/MS).

	Feed for white shrimp, ppb (µg/kg)			Feed for carp & tilapia, ppb (µg/kg)			Feed for sea bass & sea bream, ppb (µg/kg)		
Mycotoxin	**Lower**	**Moderate**	**Higher**	**Lower**	**Moderate**	**Higher**	**Lower**	**Moderate**	**Higher**
Aflatoxin B1	5	10	20	25	50	100	5	10	20
Aflatoxins	5	10	20	25	50	100	5	10	20
Ochratoxins/Citrinin	50	100	200	50	100	200	20	50	100
Type B Trichothecenes	50	100	200	250	500	1000	250	500	750
Type A Trichothecenes	15	30	60	50	100	200	50	100	200
Fusaric acid/Emerging mycotoxins	500	1000	2000	500	1000	2000	500	1000	2000
Fumonisins	750	1500	3000	2500	5000	10000	2500	5000	10000
Zearalenone	50	100	200	75	150	300	75	150	300
Other Penicillium Mycotoxins	25	50	100	40	70	100	40	70	100
Other Aspergillus Mycotoxins	20	40	60	40	60	80	20	40	60
Ergot Alkaloids	50	100	200	50	100	200	50	100	200
	Feed for rainbow trout, ppb (µg/kg)			**Feed for channel catfish, ppb (µg/kg)**			**Feed for salmon, ppb (µg/kg)**		
Mycotoxin	**Lower**	**Moderate**	**Higher**	**Lower**	**Moderate**	**Higher**	**Lower**	**Moderate**	**Higher**
Aflatoxin B1	5	10	20	25	50	100	5	10	20
Aflatoxins	5	10	20	25	50	100	5	10	20
Ochratoxins/Citrinin	20	50	100	50	100	200	20	50	100
Type B Trichothecenes	250	500	750	250	500	1000	250	500	1000
Type A Trichothecenes	50	100	200	50	100	200	50	100	200
Fusaric acid /Emerging mycotoxins	500	1000	2000	500	1000	2000	500	1000	2000
Fumonisins	2500	5000	10000	2500	5000	10000	2500	5000	10000
Zearalenone	75	150	300	75	150	300	75	150	300
Other Penicillium Mycotoxins	40	70	100	40	70	100	40	70	100
Other Aspergillus Mycotoxins	20	40	60	40	60	80	40	60	80
Ergot Alkaloids	50	100	200	50	100	200	50	100	200

Figure 1. Guideline limits for mycotoxins in aquatic species to reduce negative effects on health and performance (as specified by Alltech*). *Based on government regulations and scientific research.

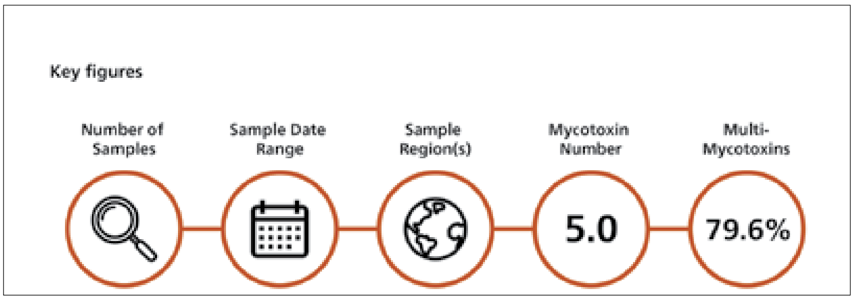

Figure 2. Results of sample analysis of aqua feeds and feedstuffs analysed by the Alltech 37+ Analytical Laboratory

Alltech perceives that good mycotoxin management provides several solutions to help farmers or feed producers mitigate the threat from field or storage mycotoxins. Its mycotoxin management program highlights mycotoxin contamination on a farm or in the feed mill, demonstrates the physical and financial impact of mycotoxin risks, and provides detailed analysis and recommendations as to the best solution to protect animal performance and profitability. One of the flagships of this program is the Alltech 37+ Analytical Laboratory which is an ISO-accredited procedure that analyses 54 mycotoxins in a sample using UPLC-MS/MS to achieve accurate determination of mycotoxins with the highest level of sensitivity.

Mycotoxin guideline limits in the feedstuffs and finished feeds specific to each individual animal species by age group and physiological status, as measured in parts per billion (ppb), are provided. These Alltech 37+® mycotoxin practical limits were established by utilising two areas of information – government regulations and scientific research. With current research, these Alltech guideline limits can be set at lower, medium or higher levels to understand mycotoxin impact on animal performance. The practical limits for aquatic animals recommended are shown in Figure 1. Furthermore, to help producers more accurately predict the effects of multiple mycotoxins on aquatic animals, Alltech Mycotoxin Management has come up with one number that takes into account the concentration and toxicity of each mycotoxin present in a sample. This value, named the Risk Equivalent Quantity (REQ), will increase when mycotoxins are combined.

The Alltech 37+ analyses thousands of samples each year. Based on this database, numerous individual mycotoxins have been detected in aquafeeds and feedstuffs as shown in Figure 2. A total of 313 samples were tested globally from year 2013 to 2020 and an average of five mycotoxins per sample were detected with about 80% of samples containing two or more mycotoxins. Figure 3 exhibits the type of mycotoxins, their average concentration and maximum levels. Based on these data, REQ risk for different species including tilapia, white shrimp and trout was assessed (Figure 4). This analysis presents the holistic picture that multiple mycotoxins truly contaminated the samples.

Another management tool, Alltech RAPIREAD™, packs a punch with rapid results, in-depth analysis and real-time recommendations. It embraces new technologies and innovations to equip farmers and feed manufacturers with the ultimate protective tool to mitigate the threat of mycotoxins. By providing actionable advice backed by data, we can now help farmers and feed manufacturers make more informed decisions to mitigate the threat from mycotoxins.

Prevention and mitigation of mycotoxins

Proper storage of grains and feedstuffs is of utmost importance to prevent fungal growth and mycotoxin contamination. Mould growth usually occurs under conditions of high moisture content (>12%), high relative humidity and in the presence of sufficient oxygen (Bruce, 2001; Clifford, 1976). Contamination of feed grains and finished fish feeds with mycotoxins can be prevented by only using feedstuffs with 12% or less moisture levels, removing debris from grains prior to storage and storing grains in clean, structurally sound bins or buildings. Feed pellets must be adequately dried and cooled to prevent mould growth. Moisture levels of less than 10% are recommended. However, despite our best efforts, mycotoxins will continue to be present in a wide range of foods and feeds.

Maximum level detected	
Aflatoxin B1:	82 ppb
Total aflatoxins:	82 ppb
Ochratoxins/Citrinin	50 ppb
Type B Trichothecenes	4572 ppb
Type A Trichothecenes	208 ppb
Fumonisins	9742 ppb
Zearalenone	1071 ppb
Fusaric acid	2586 ppb
Emerging mycotoxins	937 ppb
Other Penicillium	1682 ppb
Other Aspergillus	802 ppb
Ergot	2906 ppb
Number of samples:313	

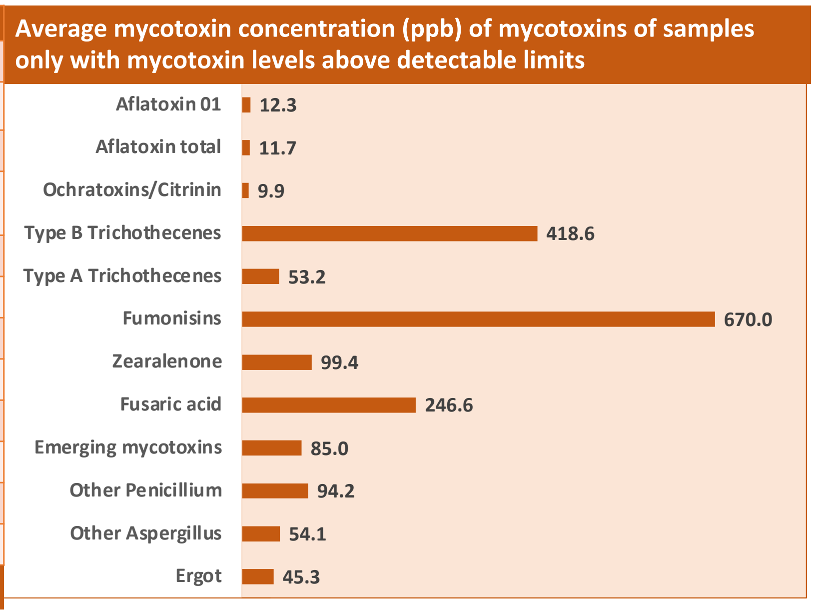

Figure 3. Average and maximum levels of mycotoxins detected in aquafeeds and feed stuffs as analysed by the Alltech 37+ Analytical Laboratory

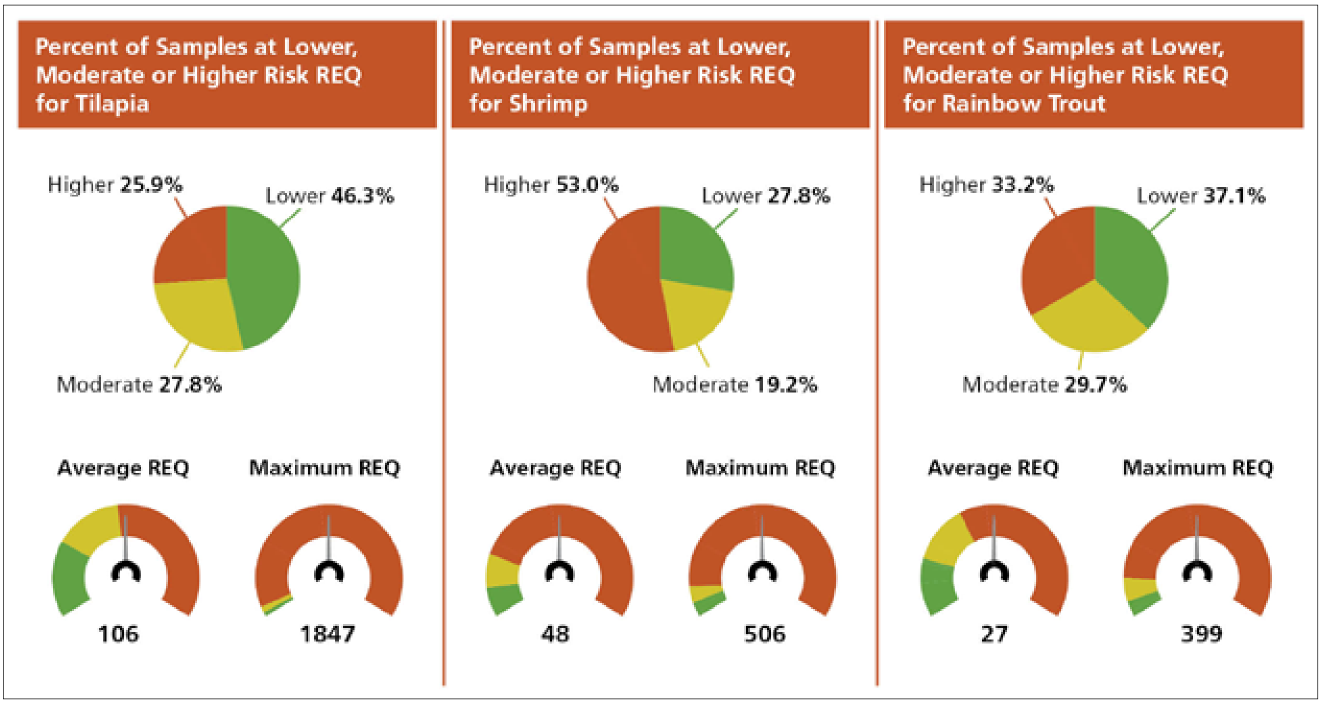

Figure 4. Total mycotoxin risk (Risk Equivalent Quantity; REQ) for selected species based on Alltech 37+ mycotoxins analysis of the feedstuffs and aquafeeds.

Consequently, strategies are required for the removal of mycotoxins from grains. Ammonia, as both an anhydrous vapour and an aqueous solution, is the detoxification reagent which has attracted the widest interest and which has been exploited commercially, by the feed industry for the destruction of aflatoxin. The ammonisation detoxification process works by chemically altering the structure of aflatoxin to form products that are not toxic to animals (Beckwith et al., 1975). However, this traditional technique is not enough to efficiently remove the aflatoxins and other mycotoxin from contaminated feedstuffs.

A more promising approach has been the use of adsorbents that bind feed-borne mycotoxins to prevent them from being absorbed by fish after consumption. There are several different adsorbent substances. Clay compounds exist in internally hydrated forms containing up to 23% moisture and appear to efficiently bind only aflatoxin, rather than other mycotoxins. However, most mycotoxin threats come from contamination from multiple mycotoxins. To deal with such contaminations, Alltech has developed Mycosorb A+ which reduces mycotoxin absorption within the animal, thereby negating the damaging effects of mycotoxins on its health and productivity. The carbohydrate components of yeast and algae cell walls in Mycosorb A+ adsorb mycotoxins due to the complementary structures with mycotoxins and are further held together by van der Waals forces and electrostatic forces between the interaction site and the mycotoxin, therefore removing the mycotoxin from the digestive tract.

Summary

Tropical aquaculture species such as carps, catfish and shrimp are exposed to mycotoxins with the increased use of plant derived raw materials in aquafeeds. With changing climatic conditions which favour the proliferation of emerging and re-emerging fungal species, the risk of mycotoxin contamination in aquaculture is higher than ever before. Proper management tools are essential to mitigate these toxins in real time. Alltech mycotoxin management tools such as Alltech 37+ and Alltech RAPIREAD help farmers and feed producers in identifying their total mycotoxin risk (REQ). Evaluating risks associated with mycotoxins on animal performance and financial losses can be more rapid than ever before. Additionally, to further manage mycotoxin risk, Mycosorb A+ reduces multiple mycotoxin absorption within the animal, thereby negating the damaging effects of mycotoxins on aquaculture health and productivity.


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Hendrix Genetics expands global shrimp breeding activities into Indonesia

With the formation of a new company, PT Kona Bay Indonesia, Hendrix Genetics will establish a broodstock multiplication centre for the benefit of the Indonesian shrimp industry. Beginning early 2021, Indonesian shrimp hatcheries will be able to source high quality, specific pathogen free (SPF) broodstock, locally reared.

Hendrix Genetics' shrimp breeding activities are well-known under the name of Kona Bay. Regular batches from the Kona Bay strategic nucleus in Hawaii will supply the Indonesian facility with the best quality production post larvae to ensure year-round availability of genetically enhanced broodstock.


Development of a broodstock multiplication centre in Ecuador.

Initial operations will be based at a SPF facility in West Java. Within two years, Hendrix Genetics plans to move to brand new state-of-the-art SPF facilities in North Bali, capable of producing over 100,000 SPF vannamei broodstock per year.

Neil Manchester, Managing Director Aquaculture of Hendrix Genetics, said, "We are delighted to announce this latest development in the expansion of our global shrimp breeding activities. Indonesia is a key market for us, and we are committed to providing all our valued customers with the very best broodstock, custom produced for the highest performance characteristics needed to support this great industry in Indonesia."

The Kona Bay brand has been leading the Indonesian market for shrimp broodstock with approximately 60% market share. With this new venture, Hendrix Genetics expects to expand its market share and volume, as the industry grows in line with forecasts. Kona Bay Indonesia is part of the strategic expansion plans of the aquaculture business unit of Hendrix Genetics.

Today, the company has shrimp breeding and production activities in Hawaii, Ecuador and Malaysia. Furthermore, the aquaculture business unit runs a trout breeding operation in the US and salmon breeding operations in Chile and Scotland. www.hendrix-genetics.com

Virbac partners with Ictyogroup to bring new aquaculture solutions

In September, Virbac announced the completion of the acquisition of the tilapia vaccines range of Ictyogroup. Virbac will now be distributing and marketing both registered and autogenous tilapia vaccines globally. Beyond the acquisition, the two companies have entered into a close partnership whereby Ictyogroup will continue to develop new vaccines and formulations for tilapia for Virbac's Aqua Division and aquatic animal health business. The deal also includes the transfer from Ictyogroup to Virbac of several managers specialised in technical and marketing support and vaccine R&D in tilapia.

Cedric Komar, CEO of Ictyopharma said, "Partnering with Virbac in the tilapia vaccine segment will clearly benefit the tilapia industry by matching Ictyogroup's vaccine R&D excellence with Virbac's worldwide marketing capabilities and customer dedication. Clearly, this deal brings the most complete and innovative tilapia vaccines to help the tilapia industry in Africa, Asia and Latin America".

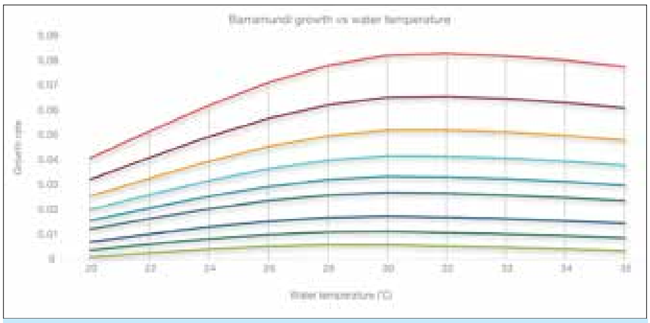
Pierre Henning, director of Virbac Aqua division added, "This deal will assemble the best of two companies committed to the development of active solutions to meet the increasing demand for aquaculture. Tilapia is the aquaculture segment enjoying the fastest growth and will be one of the solutions to help feed fast-developing populations in the medium and long term. Thanks to this portfolio acquisition and continued partnership, Virbac, which is already a significant actor in the salmon and shrimp segments will expand and complement its aquaculture operations in Africa, Asia and Latin America".

Ictyogroup is an animal health company with a strong focus on vaccine R&D. Apart from the above collaboration in the field of tilapia vaccine R&D, Ictyogroup will independently continue to develop, manufacture and market; adjuvants for the animal vaccine market (aquaculture and livestock), innovative diagnostic solutions for the aquaculture industry and a new generation proprietary fish sedative. Virbac offers veterinarians, farmers and pet owners in more than 100 countries a practical range of products and services to diagnose, prevent and treat most pathologies, while improving the animals' quality of life. www.virbac.com

Skretting launches new growth model for barramundi

The farming of the barramundi, also widely known as Asian seabass, has escalated substantially over the course of the last decade, with the fish becoming a very important marine aquaculture species throughout the Asia Pacific region. Skretting anticipates that the production of barramundi, which is increasingly being regarded as "the white salmon" due to its premium potential in the marketplace, will continue on this strong growth trend, and is committed to supporting its customers through precision services and premium feed solutions.

Utilising the comprehensive portfolio of aquaculture species' growth and feeding models that Skretting has built up over three decades, AquaSim is a unique suite of digital tools dedicated to facilitating precision farming. The latest addition to this platform is a new growth model for barramundi. Using rich data to calculate the expected farm performance, it also provides tailored feeding protocol recommendations to enable production enhancements. Crucially, this new model is suitable for the most advanced genetic barramundi strains.



Skretting's new growth model for barramundi is an industry model with realistic targets

"We worked with a large new production dataset of barramundi reared under a variety of environmental conditions both in net pens, tanks and ponds to develop this new model," explains Kristoffer Tveit, Skretting's Digital Innovation Director. "The recent data was collected from Southeast Asia, Australia and the Middle East. In addition, we had our own growth data from nutritional research trials at the Skretting ARC Hezhoubei Research Station.

"The growth model is now more robust for all growth predictions and clearly improved for large barramundi (>2 kg). Furthermore, it was interesting to see that small barramundi have a higher optimum temperature than large barramundi, which is something we have seen in other species."

"I am very pleased with the new barramundi model," says Arjen Roem, Skretting Marketing Manager South Asia. "Some growth models tend to be too theoretical in predicting maximum growth rates, and are simply unachievable in practice, but our barramundi model is an industry-model. It sets realistic feed ration targets, and it will predict growth accurately for the majority of our customers."

The new model will be part of a new precision feeding and farming service for barramundi customers offered by Skretting in the Asia Pacific region. www.skretting.com

New broodstock diet responding to hatchery needs

Launching globally in the coming months, Vitalis PRIMA is a new marine broodstock diet from Skretting that supports the health of parent fish and young fry, thereby improving the performance of hatchery systems globally. Vitalis PRIMA, which will replace Vitalis CAL, incorporates some of the latest technology to come from ground-breaking dietary research as well as invaluable commercial input from broodstock and hatchery managers. It is the latest solution to come from over 25 years of broodstock feed development at Skretting.

Using internal expertise, trials and collaborative studies, Skretting has now optimised the latest diet for several marine species, including sea bass, seabream, halibut, amberjack, turbot and cobia. At the same time, the development opened discussions with many broodstock and hatchery managers about what additional attributes they would like to see in a marine broodstock diet.

"As well as seeing increased fry survival through improved embryo vitality, hatcheries wanted diets that were much more compliant with the recirculation systems that they use, they also asked for the feed to be much more appealing to broodstock that can often stop feeding during the spawning window," says Eamonn O'Brien, Skretting Product Manager.

Five key attributes have been included in Vitalis PRIMA:

- increased fry survival through improved embryo vitality
- suitability for recirculating aquaculture systems (RAS)
- increased appeal for broodstock when feeding slows during spawning
- improved appearance of broodstock through addition of marine algae blend
- inclusion of algal oil for sustainability and stability

Vitalis PRIMA is also closely aligned with Skretting's other Vitalis diets: Vitalis Clean for lumpfish broodstock, Vitalis 2.5 for shrimp and Vitalis Repro which is offered to marine fish outside of the spawning window to maintain their optimal spawning condition.

"Vitalis PRIMA, together with Vitalis Repro, will provide a complete feeding programme for broodstock fish and make a considerable contribution to Skretting's standing as market leader in the field of broodstock nutrition. We are excited about on the long-term benefits that this will bring for farmers of marine species throughout the world," says Julio Docando-Valencia, Fish Health Diets Manager at Skretting South Europe.

DSM completes acquisition of Erber Group

In October, Royal DSM, a global science-based company in Nutrition, Health and Sustainable Living, announced the completion of its acquisition of Erber Group for an enterprise value of €980 million. The transaction – which excludes two smaller units in the Erber Group – is expected to be earnings enhancing in the first year upon completion. The acquisition of Erber Group was first announced on 12 June 2020.

DSM has acquired Erber Group's Biomin and Romer Labs. Erber Group's specialty animal nutrition and health business Biomin specialises primarily in mycotoxin risk management and gut health performance management, whereas the Romer Labs business focuses on food and feed safety diagnostic solutions. Both expand DSM's range of higher value-add specialty solutions.

The acquisition of Erber Group's Biomin further strengthens DSM's expertise and reputation as a leading provider of animal health and nutrition solutions for farm productivity and sustainability, with an emphasis on emissions reduction, feed consumption efficiency, and better use of water and land. It is therefore very much aligned with DSM's focus to make animal farming more sustainable from both an ecological and economical perspective. Romer Labs also complements DSM's human nutrition and health offering to customers in the food and beverages market segments. www.dsm.com

Aker BioMarine opens new warehouse in India

After first entering the Indian market in 2018, when Aker BioMarine India was established in Mumbai, the company has experienced growing demand for its products. The new warehouse in Chennai will strengthen local presence in India while also enabling the company to serve its customers across the region with its nutrient-rich, krill-based ingredients for fish and shrimp feeds.

"India is one of the world's largest shrimp markets, which makes it of strategic importance for Aker BioMarine. We want to be able to adequately supply our customers with our QRILL products on-demand, which is best done through local presence. That is why we have chosen Chennai for the location of our new warehouse, where we will continually stock QRILL products," said Atul Barmann, General Manager, Aker BioMarine India.

Aker BioMarine's local warehouse presence in Chennai will enable better overall service and a solid supply of buffer stock for the company's key accounts, as well as open the door to small- and medium-sized accounts looking to purchase QRILL directly.

"Our customers will benefit from a steady supply of QRILL products, from vessel to warehouse, that's delivery-ready, and already customs cleared. This increases the speed of distribution and provides easy access for customers, who can also benefit from local service and support," added Barmann.

India remains a key market for Aker BioMarine and a strategic place of operations to facilitate regional expansion for the company. The demand for krill-based ingredients for shrimp and other fish feeds in India has experienced considerable growth in recent years, and the new warehouse will provide a platform to expand the reach of QRILL Aqua products even further, beyond the borders of India. www.akerbiomarine.com

Atul Barmann

Adoption of an omega-3 rich algae into shrimp aquaculture

Thai Union Group PCL, the world's seafood leader and Corbion, the global market leader in algae-based ingredients for feed announced the expanded adoption of AlgaPrime DHA, an omega-3 rich algae feed ingredient, in Thai Union shrimp feed following a successful large scale trial in 2019. The expanded partnership marks a pivotal moment for both shrimp farmers and other seafood producers as it validates another success of using alternative, sustainable feed ingredients at scale.

The expanded adoption of AlgaPrime DHA, a native, whole algae ingredient that contains high levels of omega-3 DHA by Thai Union Feedmill PCL reflects a priority to utilise ingredients that are traceable, reliable and sustainably produced. The clean ingredient is sustainably produced through fermentation with non-GM cane sugar as a feedstock. It also helps to reduce pressure on marine resources and provides supply security for a key nutrient for shrimp growth and development.

"At Thai Union, we value driving innovation for a sustainable seafood industry and as such, we look to source new and innovative products that operationalise our SeaChange sustainability goals," said Darian McBain, Global Director of Corporate Affairs and Sustainability at Thai Union. "Our expanded work with Corbion progresses our goals on responsible sourcing by making efficient use of marine resources in aquaculture feed and complementing this with the use of alternative oils and proteins. The work with Corbion is helping to provide feed solutions that support optimal nutrition, consistency and supply chain transparency for our network of shrimp farmers."

"We are thrilled to expand adoption of AlgaPrime DHA in shrimp feed through our partnership with Thai Union," said Chris Haacke, Global Aquaculture Lead at Corbion. "AlgaPrime DHA is positioned to change the landscape of the fast-growing sustainable shrimp aquaculture industry by offering a proven solution to farmers and seafood producers. www.corbion.com

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USGC conducts research on DDGS in shrimp feed

The U.S. Grains Council set an ambitious goal in early 2020 to create 1.125 million tonnes of new demand for US distillers dried grains with solubles (DDGS) in Southeast Asia by targeting the aquafeed industry. Through a series of research projects with both public and private partners, the results are flowing in – showing economic and nutritional advantages of using the co-product in diets for shrimp and tilapia.

"DDGS show very good value in least cost formulation programs as a feed ingredient for lower crude protein feeds such as tilapia," said Ronnie Tan, USGC aquaculture consultant in Southeast Asia. "Factors including xanthophyll and phosphorus availability also may make DDGS attractive to higher value species like shrimp."

Aquaculture is a large and growing production area. In 2019, 6.5 million tonnes of tilapia and 3.8 million tonnes of shrimp were produced globally. Aquafeed for these species – specifically tilapia – is produced by modern, integrated feed mills, meaning knowledge transfer is easily translated into demand.

"The major constraint to DDGS use in aquaculture is not fear of trying, but the general unsureness of maximum allowable levels," said Caleb Wurth, USGC assistant director of Southeast Asia. "Without this data, it would be unfeasible for us to consult and promote DDGS for aquafeed."

To fill the existing knowledge gap for DDGS use in aquafeed, the Council started exploring markets in Southeast Asia for potential research partners. Indonesia was identified as a key target market due to current aquafeed demand of 1.813 million tonnes annually and aggressive domestic policy seeking to reduce dependence on wild caught fish.

The Council has partnered with the Ministry of Marine Affairs and Fisheries, Indonesia (MMAF) to examine the use of DDGS in feeding the shrimp *Litopenaeus vannamei* under Indonesian conditions. A virtual memorandum of understanding was signed this summer and the first feeding trial began in early September. In addition to this public partnership, USGC is simultaneously conducting private research with large, integrated industry partners in the region.

The Council is also working to study the value of high-protein DDGS for these specific nutritional programs. These new specialised DDGS products offer high digestibility, which adds value specifically for shrimp, Asian seabass and grouper species. The Council will continue to work with public and private partners to determine the ideal formulations for both types of DDGS for use in aquafeed.

"The accumulation of this data will help DDGS inclusion levels rise to their potential in aquafeed," Wurth said. "As consumer trends evolve, so do USGC programs to meet this shifting demand." www.grains.org

Pellets containing DDGS used in R&D trials on feeding vannamei shrimp under Indonesian conditions. Credit: Dr Romi Novriadi, MMAF, Indonesia.

In-Situ solves DO monitoring for aquaculture

RDO Blue, is the latest innovation in optical rugged dissolved oxygen (DO) technology from In-Situ. It is specifically designed to meet the DO-monitoring needs of the aquaculture industry. RDO Blue uses In-Situ's patented technology, an EPA-approved dissolved oxygen measurement method ideal for measuring DO and temperature in even the harshest environments. Suitable for handheld use or long-term deployment in fresh water or saltwater, this low-maintenance dissolved oxygen probe is particularly well suited for use in a variety of aquaculture applications, including shrimp, salmon, shellfish and catfish farming.

"As the market-leading manufacturer of optical RDO, our primary goal with RDO Blue was to maintain the sensor's performance while making it a more affordable alternative for our aquaculture customers," says In-Situ Product Manager Kate Haga. "It was essential to retain the accuracy we get with our replaceable Smart Cap and abrasion-resistant foils. But we also knew that a more cost-effective design, would be ideal for customers who require DO monitoring in multiple locations."

RDO Blue requires no calibration or conditioning prior to deployment and does not use membranes. The replaceable RDO Smart Sensor Cap stores calibration coefficients for automatic, error-free setup. A unique, three-layer system provides unmatched chemical and abrasion resistance. The High-quality Ryton construction provides enhanced reliability. An included Modbus/RS485 communication protocol supports easy integration with PLC systems and telemetry, which can be paired with In-Situ's HydroVu data services for real-time feedback and process control. www.in-situ.com

New Global Director of R&D at BioMar

BioMar has announced that from November 1, Simon Wadsworth will take over the position as Global R&D Director, heading the global R&D organisation in BioMar Group. "I am truly happy that Simon Wadsworth has decided to join us to contribute to our commitment to the aquaculture industry. He has a very experienced R&D profile and an excellent track record within R&D management, product innovation and collaboration with customers and the business. He has a highly acknowledged profile in the aquaculture industry and we are very much looking forward to having him onboard", said Carlos Diaz, CEO BioMar Group.

Simon Wadsworth has for more than 30 years been an important contributor to the aquaculture industry within feed and farming operations at Marine Harvest, Cermaq and EWOS-Cargill. He has been an important contributor to product innovations creating landmarks within nutrition and health. His last position was as Global R&D Manager in Cargill, leading the aqua R&D program.

Series C funding for insect farming startup

In October, insect farming startup Ÿnsect achieved Series C funding to USD372 million. The new capital will fund completion of the largest insect farm in the world, due to open in Amiens, France in early 2022. Ÿnsect will produce 100,000 tonnes of insect products annually.

The new funding which brought total financing to USD 425 million comes from Astanor Ventures (Series C lead investor), Upfront Ventures, FootPrint Coalition, Happiness Capital, Supernova Invest and Armat Group who joined initial Series C investors; Bpi France, Talis capital, IdInvest, Finasucre, Bois Sauvage and, Vis Vires New Protein Capital.

The startup has created a patented process for cultivating Molitor mealworm to produce a variety of highly digestible protein products to sustainably replace animal proteins for fish and livestock and pet food and fertilizers used in plant nutrition. "Our ambition is to revolutionise the food chain which, literally, starts from the basics: insects and soil. It concerns all of us, whether we are meat lovers or vegans because it is how our plants and animals are fed," said Antoine Hubert, Ÿnsect's co-founder, president and CEO. To date, Ÿnsect has USD105 million worth of contracts signed to supply customers including Skretting, the largest global fish feed company."

"Skretting is proud to be a part of Ÿnsect's success and we are confident they will continue to be a pivotal player in the global food chain for years to come," said Jenna Bowyer, Category Manager Novel Ingredients for Skretting, which has been working with Ÿnsect for the past 5 years.

Eric Archambeau, Co-Founder and Managing Partner of Astanor Ventures said that Ynsect with its proven ability to scale up and a carbon negative supply chain, is bringing a highly disruptive scalable new technology that is capable of revolutionising an important segment of a vital sector, agri-food. www.ynsect.com

Study shows algae oil can replace fish oil in farm fish

A new study led by scientists at Hubbs-SeaWorld Research Institute (HSWRI) in San Diego, California, showed that omega-3-rich algae oil can successfully replace fish oil in the diet of farm-raised juvenile California yellowtail (*Seriola dorsalis*) without compromising growth or survival. The results also revealed that algae oil can produce fish with levels of two important omega-3 fatty acids—DHA and EPA—roughly three times higher than in fish fed fish oil. These findings, published in the journal *Aquaculture Research*, put scientists steps closer to growing healthier and more sustainable seafood by replacing wild-caught fish in aquafeed.

"These results show the potential for this species to be reared on fish meal and fish oil free diets," said Kevin Stuart, a research scientist at HSWRI and lead author of the study. "More research needs to be done to make these diets cost effective for growers, but the potential is there to advance sustainable aquaculture."

In HSWRI's 64-day feeding trial, the researchers tested three diets formulated without wild fish ingredients against a control diet—containing fish meal and fish oil—on juvenile California yellowtail. Upon completion of the study, the fish were analysed for general body composition and fatty acid levels in their tissue to evaluate the fish growth and fillet quality of each diet.

The researchers found significantly higher DHA-EPA fatty acid levels in fish fed the diet containing Veramaris® marine algal oil, which contains naturally high levels of these omega-3s. Result demonstrate that natural marine algal oil can fully replace fish oil in the diet of juvenile California yellowtail without affecting growth or survival. In previous studies on Pacific white shrimp (*Litopenaeus vannamei*), channel catfish (*Ictalurus punctatus*), rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*), scientists have observed similar or better growth and higher levels of omega-3 fatty acids in fish fed algal oil when compared to those fed fish oil.

Although the diets free of wild fish ingredients resulted in slower fish growth than the fish meal-fish oil control diet, the pattern of growth—600% increase in weight during the study—was still excellent and suggests a strong potential for full fish meal-fish oil replacement for this species with continued research, said the authors.

Farm raised California yellowtail at Hubbs-SeaWorld Research Institute. Credit: Hubbs-SeaWorld Research Institute

Greater potential for natural genetic variation to boost production benefits

Well-designed genetic selection programs can result in major gains in fish production. However new research into Atlantic salmon, by Dr Alicia Bertolotti, has highlighted a hitherto untapped resource of genetic variation that could help unlock even greater production benefits.

Structural variation is another major class of genetic variation, where large sections of the genetic code can be duplicated, inverted or even completely absent comparing different individuals.

As structural variations are passed from one generation to the next, they provide another potentially valuable tool to use when maximising production gains for fish farmers. Recent breakthroughs in computing power, bioinformatic algorithms and improvements in genetic sequencing technology have made it possible to identify structural variants, though this remains challenging.

Bertolotti's research involved sequencing the genomes of 492 Atlantic salmon in a project led by Professor Dan Macqueen at the Roslin Institute (University of Edinburgh) in partnership with Xelect, the University of Aberdeen and many international collaborators. This team included the Norwegian University of Life Sciences as a key collaborator, providing much of the sequencing data among other important contributions. It was the first major study into structural variants in any farmed fish and the results have been published in the journal Nature Communications. The paper is a key output from Bertolotti's PhD, completed at Aberdeen, under the industrial CASE scheme of the UK's Biotechnology & Biological Research Council (BBSRC). Xelect acted as industry partner, contributing funding, a work placement and access to its large archive of DNA samples and trait data. Xelect CEO Professor Ian Johnston co-supervised the doctorate.

"One of the greatest challenges we faced was filtering out the many false variants that were not true structural variations. None of the automated systems were accurate enough, so in the end we did it manually, which was an enormous task," said Bertolotti. One particularly interesting finding was that many structural variations were in brain-expressed genes that influence behaviour. Farmed salmon appear to have accumulated more of these variants than their wild relatives, presumably because of selective breeding for domesticated strains. There is clearly considerable potential for structural variations to increase trait gains using natural genetics.

Xelect's Operations Director, Tom Ashton said, "Alicia's PhD and the resulting paper in Nature Communications represent an important step forward in aquaculture genomics, bringing us closer to being able to harness the power of structural variations in selective breeding programmes". www.xelect-genetics.com

VNU announces new schedule for 2021

Due to the COVID-19 pandemic, travel restrictions, and ongoing worldwide uncertainty, VNU Group has modified its show calendar to secure successful inter-regional exhibitions during the second half of 2021. The schedule modifications will result in the postponement of six leading events in the agribusiness sectors, organised by VNU Asia Pacific, VNU Europe, and partners. The events were originally planned to take place in the upcoming months in Southeast Asia.

"Our exhibitions mobilise and connect cross-regional markets. High-quality visitors from several economies is the client expectation for these shows. This is especially true with VIV Asia, our flagship event for the Feed to Food portfolio and the leading platform of its kind throughout the entire Asia-Pacific region" said Heiko M. Stutzinger, Managing Director of VNU Asia Pacific and Director VIV worldwide/VNU Europe.

"After consultation with our esteemed co-organising partners, exhibitors, and respective venues, we have decided to take action in advance and postpone VIV Asia, Meat Pro Asia, Free From Food Asia, ILDEX Vietnam, ILDEX Indonesia and Aquatica Asia until the second half of 2021" announced Stutzinger.

Originally planned for March, VIV Asia and the newly co-located events, Meat Pro Asia and Free from Food Asia are now scheduled for **September 22-24, 2021, Bangkok, Thailand**.

"Global travel restrictions have remained and Thailand continues to enforce a mandatory 14-day quarantine for all visitors entering the country. Our local office in Bangkok is monitoring the cautious measures that the Thai government is taking due to concern of a second COVID-19 wave, as witnessed in other countries" stated Panadda Kongma, Director of VNU Asia Pacific.

"VIV Asia alone attracts more than 50,000 professionals with 70% of participants from outside of Thailand. As organisers, we believe that a high-quality show with an expected number of international buyers can come to fruition by postponing the shows until September 2021" explains Zhenja Antochin, Senior Project Manager of VNU Europe.

VNU Asia Pacific and partners have also decided to reserve new dates for the following:

- ILDEX Vietnam is postponed to July 21-23, 2021 at the Saigon Exhibition and Convention Centre (SECC), Ho Chi Minh City, Vietnam.
- ILDEX Indonesia and Aquatica Asia is postponed to November 24-26, 2021 at the Indonesia Convention Exhibition (ICE), Jakarta, Indonesia.

The adjusted show calendar allows for adequate preparation for all concerned stakeholders. VNU is determined to deliver high-quality shows without compromising the inter-regional nature of the events. www.viv.net; www.ildex.com; www.vnuasiapacific.com

AQUA CULTURE Asia Pacific in 2021

Volume 17	Jan/Feb	Mar/Apr	May/Jun	Jul/Aug	Sep/Oct	Nov/Dec
Aqua Business *Feature articles from industry players*	Experiences and opinions covering role models and clear and present needs of industry					
Issue focus Recent developments/spotlight on emerging challenges	Nursery & Hatchery	Health & Disease Management	Demand & Supply Equilibrium	Sustainable & Responsible Aquaculture	Aquaculture Innovations	Health & Disease Management
Industry Review Developments, outlook, demand & supply	Marine Shrimp	Marine Fish	Aquafeed Production	Tilapia	Marine Shrimp	Catfish & Freshwater Fish
Feeds & Processing Technology Technical contributions from industry	Larval & Nursery Feeds	Novel Ingredients/ Fish meal/oil Replacements	Extrusion and Pelleting	Sustainable Feeds	Functional Feeds/Additives	Feed Enzymes/Post Pellet Applications
Production Technology Technical information along the value chain	Controlled Systems (hybrid/RAS)	Offshore and Industrialisation	Hatchery Technology	Real Time Monitoring/Big Data	Feed management	Post-Harvest Processing
Marketing activities	Market and product developments, market access, certifications, branding, food safety etc					
Company/Product News	News on activities at international, regional and local conferences and trade shows					
Deadlines -*Technical articles*	***Nov 13, 2020***	***Jan15***	***Mar 12***	***May 14***	***Jul 16***	***Sep 16***
Deadlines - *Advert Bookings*	***Nov 20, 2020***	***Jan 22***	***Mar 19***	***May 21***	***Jul 23***	***Sep 23***
Events ***Distribution at these events as well as local and regional meetings*** ****Show preview***			***World Aquaculture 2020** ***Singapore*** *Jun 14 – 18*	***TARS 2021: Shrimp Aquaculture** ***Ho Chi Minh City, Vietnam*** *Aug 19 – 20*	**VIV Asia 2021** ***Bangkok, Thailand*** *Sep 22-24*	
		Seafood Expo Global 2021 ***Barcelona Spain*** *Apr 27 – 29*		**DAA11** ***Kuching, Malaysia*** *Aug 23 – 26*	**Aquaculture *Europe 2021*** ***Madeira, Portugal*** *Oct 4 – 7*	
				Asian-Pacific Aquaculture 2021** ***Surabaya, Indonesia *Sep 7 – 10*	**RAStech 2021** ***South Carolina, USA*** Nov 3 – 4	

For article contributions, please contact editor: zuridah@aquaasiapac.com

World Aquaculture 2020 Webinar Series

The World Aquaculture Society will conduct a free webinar for WA2020 participants during December 14-16, 2020 on important aquaculture topics based on the theme "Next Generation Aquaculture, Innovation and Sustainability will Feed the World". Each day, the webinar will be from 14.00-16.00hrs SGT (GMT +8.00). The program is given below and register at www.was.org

December 14, 2020 (Plenary Session)
Matthias Halwart - *Adapting to change - lessons from regional reviews on aquaculture*
Farshad Shishehchian - *Aquaculture technologies & super intensive culture system to create food security.*
Panellists: Leong Hon Keong, Guillaume Drillet and presenters

December 15, 2020
Session I: Addressing the disease challenges in shrimp aquaculture
Luis Fernando Aranguren Caro - *EHP/White faeces syndrome*
Kallaya Sritunyalucksana - *Response to the threat of DIV1 in shrimp aquaculture*
Panellists: Prof Dato Mohamed Shariff, Rohana Subasinghe and presenters

Session II: Seafood Safety
John Michael Wigglesworth - *ASC certification on seafood*
Guillaume Drillet - *Plastics in Aquaculture*
Panellists: Diana Chee and presenters

December 16, 2020
Session I: Production technology focus - RAS, novel offshore and barge systems, automation
Leow Ban Tat - *Opportunities for floating closed containment systems for fish farming*
Romi Novriadi - *Production technologies and recent tech developments in the shrimp industry in Southeast Asia*
Panellists: Dean Jerry and presenters

Session II: Nutrition: Fish and shrimp
Brett Glencross - *Advances in nutrition for tropical marine fish*
Albert Tacon - *Aquaculture nutrition & health*
Panellists: Jennifer Cobcroft, Prof Mohd Salleh Kamarudin and presenters

EAS will organise AE2020 ONLINE!

The EAS Board has decided that the Aquaculture Europe 2020 event planned in Cork next April cannot now go ahead. It has decided to organise AE2020 as an ONLINE event, held over the same dates April 12-15, 2021. The basic format of the event will stay the same as 'normal' Aquaculture Europe meetings, with morning plenary sessions and then breakout parallel sessions for oral and Eposter presentations. The parallel sessions will have shorter slots for pre-recorded video presentations of the oral presentations. The deadline for submission of abstracts is extended to January 31. There will be an e-Market, where vendors and media partners will have a dedicated place online to present their products, link to their website and have a chat-box to interact with attendees and set up meetings.

Gavin, Burnell, outgoing EAS President and AE2020 Steering Committee chair said that "Taking this decision now, 6 months ahead of the date, will allow us to prepare and configure the event so that it can be successful and interactive for attendees. This early announcement and the extension to the abstract deadline will also assure authors that they will have a chance to present their work and will position AE2020 against the many other online events taking place this year and early next".

Parallel sessions with pre-recorded presentations will not only reduce the risk for technical problems but ensure the smooth running of sessions. All presentations (oral videos and Eposters) will be available for a period after the event for attendees to see online. During that period, contact with the presenter will also be facilitated, complementing the live Q&A during the conference days.

EAS will also organise the planned RAS@EAS event as a one-day online workshop on April 12. The workshop theme "Creating an Optimum Environment" will be a panel based discussion organised in three sessions addressing three key questions:

- How do we best approach disinfection?
- Where are we going with monitoring & autonomy?
- What are the most problematic interactions between fish & their environment?

Registration for this event can be made separately, but all those registered for the full AE2020 event will be able to attend. During the opening plenary session, there will be the EAS Student Spotlight Award, with short pitches from the finalists and where participants will decide the winner. www.aquaeas.org

Appointment

Broodstock Sales Manager for India

Durgaprasad SV has been appointed as Broodstock Sales Manager- India for American Penaeid (API) Company, Florida, USA. He has over 26 years of experience in the shrimp farming industry including positions with Goldcoin Biotechnologies (Malaysia) and Marine Leader Co Ltd (Thailand). Along with this new position, Durgaprasad will also represent CPAC Asia Animal Health (Thailand).

Announcement on new dates

Rescheduled to June 6-11, 2021
The local organising and international scientific committee of **International Symposium on Fish Nutrition and Feeding (ISFNF)** has rescheduled the event. The meeting will be held at BEXCO, Busan, Korea on **June 6-11, 2021**. Changes to deadlines are as follows:

Early registration - March 19
Abstract Submission - April 16

Organisers will continue to monitor the global spread of Covid-19. In addition, the LOC is discussing the possibility of an online version of ISFNF 2020.

The aim of ISFNF is to advance all aspects of aquatic animal nutrition research. It brings together researchers, industry, government and other stakeholders to identify and address constraints limiting aquaculture production, fish health and food safety, with a view to recognising the need for adopting approaches based on new knowledge, emerging technology and novel ideas.

More information: www.isfnf2020busan.com/

Postponed to August 23 – 26 2021,
After considering the concerns related to the health and safety of participants, sanitary measures and border controls that have been or could be put in place by the host countries and other governments, and restrictions caused on air travel; the organising committee of the **11th Symposium on Diseases in Asian Aquaculture (DAA11)** announced that it could not envisage a face-to-face symposium for the rescheduled date in September/October 2020.

Therefore, the Government of Malaysia and the DAA11 organising committee agreed to reschedule DAA11 to **August 23 – 26, 2021** in Kuching, Sarawak. They added that all papers, panels, posters, roundtables that have been accepted to DAA11 2020 will be automatically accepted for DAA11 2021. New deadlines are as follows:

Registration and Abstract Submission - January 10
Abstract Submission - March 31
Notification of Abstract Acceptance - June 1
Early Registration - June 30
Normal Registration - July 15

More information: www.DAA11.org; Email: daa11@dof.gov.my (DAA11 secretariat).

2021

Details on the events below are available online at **http://www.aquaasiapac.com/news.php**
To have your event included in this section, email details to **zuridah@aquaasiapac.com**

March 24-26
VietShrimp Aquaculture International Fair
Cantho City, Vietnam
https://vietshrimp.net

April 12-15 (online)
Aquaculture Europe (AE2020 Cork)
Cork, Ireland
https://aquaeas.eu/

April 27-29
Seafood Expo Global 2021
Barcelona, Spain
https://www.seafoodexpo.com/global/

June 6-11
ISFNF 2020
Busan, Korea
www.isfnf2020busan.com

June 14-18
World Aquaculture 2020
Singapore
www.was.org

August 18-19
TARS 2021: Shrimp Aquaculture
Ho Chi Minh City, Vietnam
www.tarsaquaculture.com

August 23-26
11th Symposium on Diseases in Asian Aquaculture (DAA11)
Kuching, Malaysia
www.daa11.org

August 24-26
Livestock Malaysia 2021
Melaka
www.livestockmalaysia.com

August 25-27
Vietfish 2021
Ho Chi Minh City
www.vietfish.com.vn

September 7 - 10
Asian-Pacific Aquaculture 2021
Surabaya, Indonesia
www.was.org

September 14-17
SPACE 2021
Rennes, France
www.space.fr

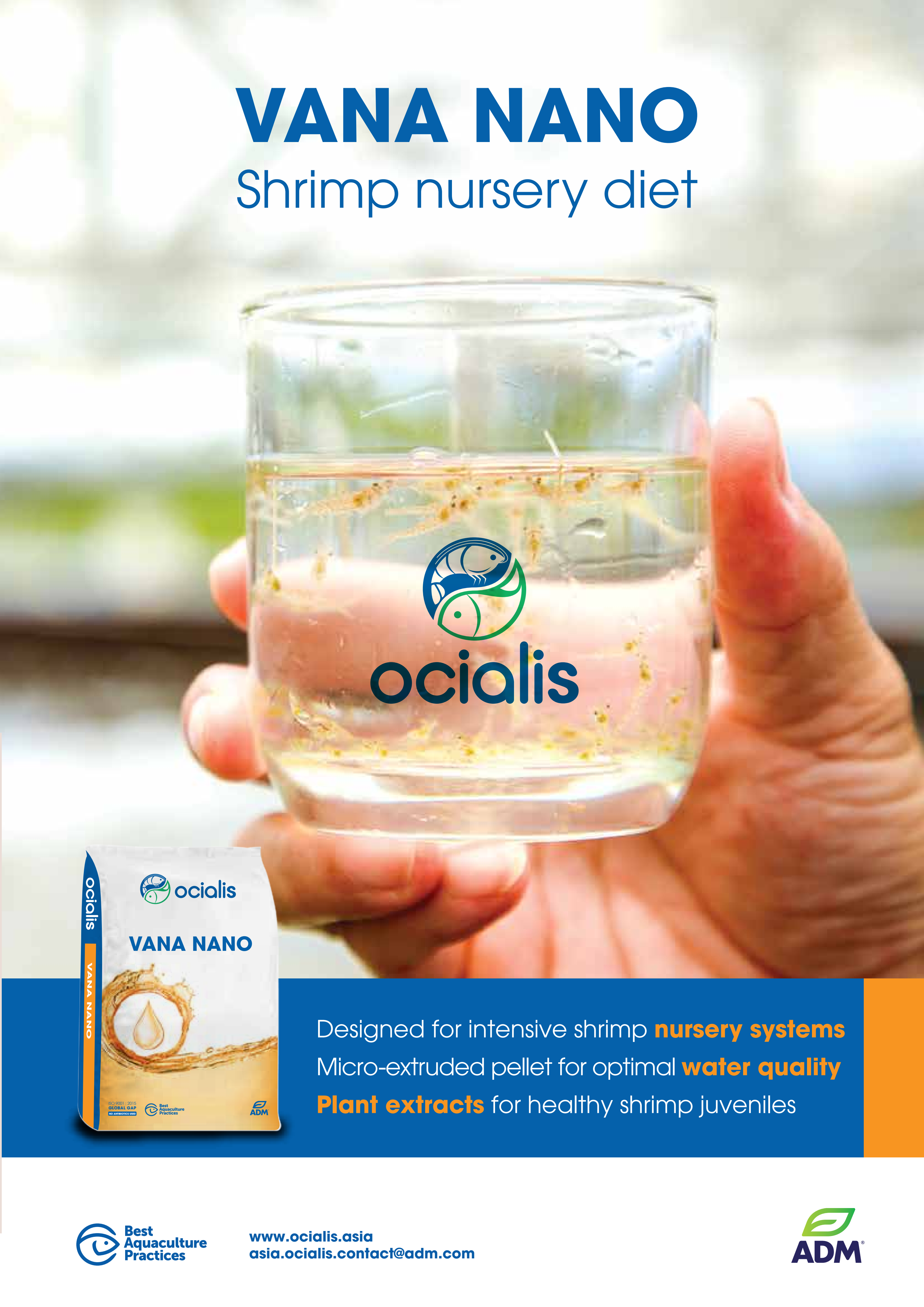
September 22-24
VIV Asia
Bangkok, Thailand
www.viv.net

October 4-7
Aquaculture Europe (AE2021)
Madeira, Portugal
www.aquaeas.org

November 3-4
RAStech
South Carolina, USA
www.ras-tec.com

November 15-19
World Aquaculture 2021
Mérida, Mexico
www.was.org

December 10-13
AFRAQ20
Alexandria City, Egypt
www.was.org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